# LIPID-BASED ASSESSMENTS OF OMNIVORY IN ARCTIC COPEPODS



CATHERINE JOY STEVENS

# LIPID-BASED ASSESSMENTS OF OMNIVORY IN ARCTIC COPEPODS

By

*Catherine Joy Stevens*

A thesis submitted to the School of Graduate Studies

In partial fulfillment of the requirements for

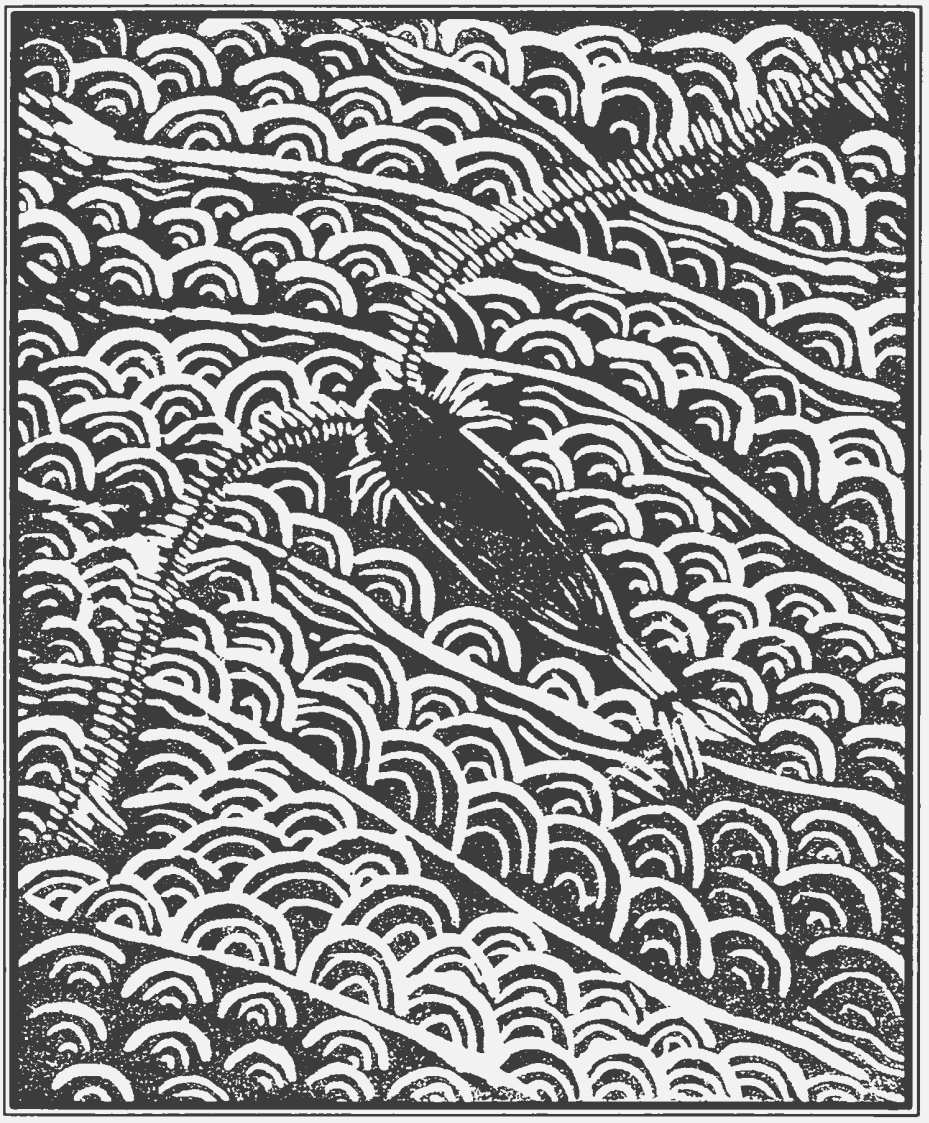
The degree of Doctor of Philosophy

Department of Biology, Faculty of Science

Memorial University of Newfoundland

December 2003


St. John's Newfoundland



## Thesis abstract

The lipid compositions of three dominant arctic copepods, *Calanus hyperboreus*, *C. glacialis* and *Metridia longa*, were used to deduce feeding strategy (i.e., degree of omnivory) and diet in the highly productive North Water Polynya during autumn. A quick way of processing lipid samples, well suited to fieldwork in remote regions, was tested against conventional methods. The quick method, in which copepods were frozen together and sorted later, provided accurate estimates of lipid classes in *Calanus hyperboreus* CV and fatty acids in *Metridia longa* females. However, it was inappropriate for sensitive analyses of certain biomarkers in the former species and lipid classes in the latter.

A new omnivory index, the unsaturation coefficient (UC), was tested during a long-term incubation with *Calanus glacialis* CV. The UC is the ratio between polyunsaturated and total wax ester (WE). The WE fraction of three arctic copepods split according to the degree of acyl lipid unsaturation during non-polar chromatographic development. UC and diatom biomarker levels decreased significantly after copepods were fed a bacterivorous dinoflagellate for three weeks. At the same time, proportions of the bacterial biomarker 18:1(n-7) increased significantly. When copepods were reintroduced to a diatom diet, their lipid profiles reversed to a more herbivorous signature over a period of three weeks, characterized by higher UC levels.

In several regions of the polynya, *Metridia longa* females had significantly lower UC than *Calanus hyperboreus* CV and *C. glacialis* CV. *M. longa* contained high proportions of triacylglycerols, polar lipid and 18:1(n-9), and low relative amounts of

WE, polyunsaturated fatty acids, 20:1(n-9) and 22:1(n-11). UC and previously established determinants of feeding strategy were in agreement and showed that *M. longa* fed more omnivorously than the two *Calanus* species. Principal components analysis revealed that all three species were feeding omnivorously, to varying degrees, in the southeastern (SE) polynya. Copepods here contained low proportions of diatom, phytoplankton and herbivory indices and had low UC, as compared to those at northwestern stations (NW). Instead, copepods in the SE generally had elevated levels of carnivory, dinoflagellate and bacterial markers. Spatial patterns in seston lipids and other data indicated that the microbial loop was more active in the SE than in the NW.

## Acknowledgements

Firstly, I thank my supervisor Dr. Don Deibel for providing an opportunity to participate in an exciting international project (*The International North Water Polynya Study*) and to join a vibrant laboratory and scientific community at the Ocean Sciences Centre. Don's love of science, curiosity, and sense of humor were contagious and very important in the development of my ideas and practical approach. Dr. Chris Parrish provided a wealth of important chemical and methodological knowledge that was invaluable in the completion of this thesis. I also appreciated the support and suggestions from my supervisory committee members, Drs. Ray Thompson and Paul Snelgrove. Everyone at the OSC, especially the secretaries, shuttle-man Jim, the shop guys, the divers, and the technicians, made it a great place to work.

Many friends deserve special attention for making my experience all the more enjoyable. I'd like to thank Louise Copeman for the chloroform-induced hilarity, Patty Saunders for friendship and advice, Sing-Hoi Lee (Mon Amour) for his pesky-yet-endearing fatherly concern, and all the members of the Deibel and Parrish labs for being so helpful and friendly. Thanks to my fellow graduate students for all the giggles and commiseration. I also thank my family, who has been incredibly supportive from afar. I'd like to finish by thanking my husband Stéphane. This journey would have been much more difficult without his love, friendship and support (not to mention silly antics and stress-reducing tickle fights). *Reproductions of the original linoleum block cut "Copepod" used with permission of the artist (© Brian A. Stevens, Roslin, Nova Scotia, 2003).*

## Table of contents

## List of Tables

## List of Figures

## List of abbreviations and units (where applicable)

| | |
|---|---|
| ALC | Alcohol |
| AMPL | Acetone-mobile polar lipid |
| ARA | Arachadonic acid (20:4(n-6)) |
| C:Chl | Carbon:chlorophyll |
| CA | Cluster analysis |
| CCAP | Culture Collection of Algae and Protozoa (Oban, UK) |
| CI | Carey Islands |
| CNBB | Central Northern Baffin Bay |
| CTD | Conductivity, temperature, depth |
| CIII | Copepodite stage 3 |
| CIV | Copepodite stage 4 |
| CV | Copepodite stage 5 |
| CVI | Copepodite stage 6 (adult) |
| CVI-F | Adult female |
| cv | Coefficient of variation |
| DG | Diacylglycerol |
| DHA | Docosahexaenoic acid (22:6(n-3)) |
| DM | Dry mass (mg) |
| EPA | Eicosapentaenoic acid (20:5(n-3)) |
| FFA | Free fatty acid |
| F-S | Freeze-sort |

| | |
|---|---|
| FID | Flame ionization detector |
| GC | Gas chromatograph |
| HC | Hydrocarbon |
| KET | Ketone |
| ME | Methyl ester |
| MIZ | Marginal ice zone |
| MUFA | Monounsaturated fatty acids (1 double bond) |
| ncp | No copepods present |
| nd | Not detected |
| NOW | North Water Polynya |
| nr | No relationship |
| ns | Not statistically significant |
| NWA | North Water Assembly |
| OBFA | Odd-numbered and/or branched-chain fatty acids |
| PAR | Photosynthetically available radiation |
| PC | Principal component |
| PCA | Principal components analysis |
| PL | Phospholipid |
| POC | Particulate organic carbon ($\mu g\ l^{-1}$) |
| PUFA | Polyunsaturated fatty acids ($\geq$ 2 double bonds) |
| SA | Southern Assembly |
| SE | Steryl ester |

| | |
|---|---|
| S-F | Sort-freeze |
| SFA | Saturated fatty acids (no double bonds) |
| S-NWA | Southern-North Water Assembly |
| SS | Smith Sound |
| ST | Sterol |
| TFA | Total fatty acids (μg copepod$^{-1}$; ng ml$^{-1}$ (seston)) |
| TG | Triacylglycerol |
| TL | Total lipid (sum of lipid classes: μg copepod$^{-1}$: ng ml$^{-1}$ (seston)) |
| tr | Trace (< 0.1%) |
| UC | Unsaturation coefficient (dimensionless) |
| WE | Wax ester |
| WE-I | Wax ester peak I |
| WE-II | Wax ester peak II |
| WGC | West Greenland Current |
| $Z_{1\%}$ | 1% light (euphotic) depth (m) |
| $Z_m$ | Mixed layer depth (m) |

## List of Appendices

# Chapter 1. Introduction and overview

## 1.1 The role of omnivory in food webs

The existence, extent, and consequences of omnivory have been intensely debated for decades (Polis and Strong 1996; Closs et al. 1999). Early theoretical investigations suggested that omnivory was destabilizing: food web models incorporating omnivory took longer to recover from perturbations than those without (Pimm and Lawton 1977; 1978). Increased food web complexity, including species diversity and numerous trophic levels characterized by weak connections, was also deemed unstable in these works. Furthermore, it was argued that in nature, omnivory was uncommon and most food webs were simplistic chains composed of few and discrete trophic levels. Both empiricists and theoreticians have since disagreed with these claims.

Many now concur that food webs are reticulate and omnivory is common, a claim for which much supporting empirical evidence has developed (Polis and Strong 1996; Closs et al. 1999). A consistently high degree of omnivory was observed within zooplankton communities during an extensive survey of over 500 North American lakes (Sprules and Bowerman 1988). Recent models, based on more biologically realistic assumptions, suggest that food web complexity and omnivory contribute to community persistence (Sterner et al. 1997; McCann et al. 1998). In a rare study, the effect of omnivory on food web stability was investigated directly by manipulating arthropod communities in the blowdown zone of Mount Saint Helens (Fagan 1997). He found that

in the face of external perturbation, omnivory helped stabilize the community by preventing decreases in arthropod densities.

In some cases, omnivory implies a certain degree of cognizance on the part of the consumer. For example, animals ranging from protozoans to birds actively switch between prey, disproportionately consuming that which is in greatest abundance (Oaten and Murdoch 1975). These switches are thought to be largely behavioral, mediated by feeding history and prey refugia. Switching behaviour often follows Holling type III kinetics (sigmoidal). This particular functional response may promote prey diversity and survival due to the presence of a threshold at low densities and density-dependent consumption at high prey concentrations (Murdoch and Oaten 1975). Interestingly, a type III curve may also arise as a result of optimal foraging (Lehman 1976). One assumption of optimal foraging theory invokes switching: consumers that optimally forage are selective, particularly when prey is plentiful (Hughes 1980). Models show that "adaptive foragers", such as those that switch, are essential to community persistence in complex food webs (Kondoh 2003). Without them, complexity is destabilizing.

## 1.2 Copepod omnivory in the marine environment

Reports of copepod omnivory date back to qualitative observations made during the Challenger expedition (see Gifford 1991). However, omnivory was rarely quantified until the importance of microbes in marine systems was recognized 20 to 30 years ago (Pomeroy 1974; Azam et al. 1983). To this end, the earliest estimates of ingestion rates

on non-phytoplankton material (ca. 1980s) were done *in vitro* using copepod eggs, nauplii, faecal pellets and detritus as prey (Poulet 1983; Kleppel 1993). Later, a strong link between copepods and protozoans was proposed (Stoecker and Capuzzo 1990; Gifford 1991). It was suggested that protozoans were an important alternate prey for copepods because of their abundance in nature, and their size, motility, and high nutritional content. The first quantifications of protozoan ingestion by copepods under simulated *in situ* conditions were for *Acartia* spp. from near-shore environments (Gifford 1991). Despite these reports, other temperate species were still considered strict herbivores. This perception was especially true for *Calanus* spp., long known to represent crucial links between seasonal diatom blooms and many commercial fisheries (Runge 1988; Cushing 1989).

*In situ* ingestion of protozoans by *Calanus* spp. has since been quantified in the Arctic (Levinsen et al. 2000), Antarctic (Atkinson 1995; Atkinson 1996; Froneman et al. 1996), North Atlantic (Ohman and Runge 1994; Gifford et al. 1995) and in mesocosms (Nejstgaard et al. 1997; Nejstgaard et al. 2001). These studies also revealed that protozoans are often preferred over phytoplankton, they yield higher secondary production rates in mesocosms than do diatoms, and they can sustain egg production during oligotrophy. Quantitative reports of omnivory have weakened the copepod-diatom link in the classical food chain; it has been replaced by an alternate paradigm in which copepods are strongly featured in the microbial food web (Sherr et al. 1986; Fenchel 1988; Sherr and Sherr 1988; Gifford 1991; Kleppel 1993).

Copepods can be highly selective feeders, discriminating between prey on the basis of size, quantity, quality (including "taste", nutritional content and toxicity) and motility (Poulet 1983; Huntley 1988; Kleppel 1993; Harris 1996). These choices may be behavioral. Under controlled laboratory conditions, copepods employ switching behavior when offered a mixture of motile and non-motile prey (Landry 1981; Kiørboe et al. 1996; Gismervik and Anderson 1997). Copepods feed disproportionately on the prey in greatest abundance, actively ambushing nauplii and ciliates, and passively consuming diatoms via suspension feeding. These behavioral mechanisms were proposed in other studies with copepods (Jørgensen 1966; Richman and Rogers 1969; Greene 1988). It has been suggested that copepods switch between prey to maximize energy gain (i.e., optimal foraging; Lehman 1976; Kiørboe et al. 1996) and under these conditions, they respond to food according to type III kinetics (Gismervik and Anderson 1997). As "adaptive foragers" (after Kondoh 2003), omnivorous copepods may contribute to community stability. Switching behavior is a possible mechanism behind dietary patterns observed in nature where copepod diets and feeding rates are strongly related to seasonal changes in prey composition (e.g., Ohman and Runge 1994; Gifford et al. 1995; Levinsen et al. 2000).

### 1.3 Omnivory assessment tools

A lack of robust methods continues to hamper quantitative studies of copepod omnivory under natural conditions. At present, we lack a true *in situ* method that allows simultaneous determination of ingestion rates on heterotrophic and autotrophic prey.

Prey-specific ingestion rates are best obtained through incubations of grazers with food where food cells are identified and counted by microscopy. Even when natural assemblages of copepods and prey are used (i.e., "simulated" *in situ* design), various containment effects can occur (e.g., Roman and Rublee 1980; Saunders et al. 2003) and information obtained in this way may reflect experimental artifacts.

Attempts to study omnivory *in situ* have led to the development and use of biochemical markers and other tools for distinguishing prey. Floristic analysis of material in copepod guts (Hopkins 1987; Hopkins and Torres 1989) and faecal pellets (Urban et al. 1993) can demonstrate omnivory, but the degradation of soft-bodied protozoans during digestion and sample preservation is a major caveat (Stoecker and Capuzzo 1990; Gifford 1991). The use of pigments specific to heterotrophic prey (e.g., astaxanthin, canthaxanthin) as omnivory indices has been unsuccessful (Juhl et al. 1996). Stable isotope analysis (e.g., Hobson et al. 2002) helps decipher food webs via estimates of relative trophic positions, but it cannot yield detailed dietary information.

Lipid biomarkers, especially sterols and fatty acids, are being used increasingly to study trophic dynamics in marine food webs. Certain sterols are diagnostic of different microplankton groups (Volkman et al. 1998), but overall sterol diversity decreases exponentially as material moves between trophic levels (Parrish et al. 1996; Yang et al. 1996). Therefore, sterols are not particularly useful for determining the natural diets of higher order consumers such as copepods. In the field of copepod ecology, fatty acid

biomarkers have been extensively and successfully used to determine feeding strategy (i.e., degree of omnivory) and diet (Falk Petersen et al. 1987; Kattner et al. 1989; Graeve et al. 1994a; Cripps and Hill 1998). Fatty acids are suitable dietary biomarkers because they can differentiate between prey groups (Ackman and Tocher 1968; Volkman et al. 1989; Viso and Marty 1993) and are conservative (Lee et al. 1971). Because these compounds have long residence times in copepod tissues (Graeve et al. 1994b), *in vivo* compositions represent integrated, ecologically meaningful approximations of assimilated food. Fatty acids, particularly the 'essential' fatty acids 20:5(n-3) (eicosapentanoic acid; EPA), 20:4(n-6) (arachadonic acid, ARA), and 22:6(n-3) (docosahexanoic acid; DHA) are crucial for optimal zooplankton growth and development (Brett and Müller-Navarra 1997; DeMott and Müller-Navarra 1997).

Certain fatty acids are considered diagnostic of diatoms (e.g., 16:4(n-1)), dinoflagellates (e.g., 22:6(n-3)) and prymnesiophytes (e.g., 18:5(n-3)) (Sargent et al. 1985; Viso and Marty 1993). Odd-numbered and/or branched-chain fatty acids and (n-7) and (n-9) monounsaturates (e.g., 18:1(n-7)) characterize bacteria (Sargent et al. 1987; Kaneda 1991; Pranal et al. 1996 and references therein). They have been used successfully to infer *direct* ingestion of microbial material by crabs (Meziane and Tsuchiya 2000), gastropods (Pranal et al. 1996), bivalves (Zhukova et al. 1992), freshwater cladocerans (Desvilettes et al. 1994) and polychaetes (Meziane et al. 1997). The utility of these biomarkers in establishing connections between pelagic copepods and the microbial food web has not been determined.

## 1.4 The importance of Arctic polynyas

Polynyas are recurring areas of open water bounded by ice. They often support large populations of marine mammals and seabirds (Stirling 1997) and have thus been likened to oases in the desert. Polynyas are thought to be key in the early development of intense diatom blooms that support large zooplankton populations and hence the higher trophic levels of the arctic food web (Stirling 1997). It is also believed that polynyas are sites of significant $CO_2$ sequestration (Yager et al. 1995). Polynyas represent model environments in which to investigate climate change. Their very design simulates global warming and changes in their properties (e.g., size and frequency) may act as early warning signals. Such insight requires intensive study of the physical, biological and chemical characteristics of polynyas.

The North Water, located between Ellesmere Island (Canada) and Greenland (Denmark), is one of the largest and most productive polynyas in the world (Deming et al. 2002; Klein et al. 2002). Several key hypotheses and questions were addressed between 1997 and 1999 as part of the *International North Water Polynya Study*, the first multidisciplinary large-scale study of this ecosystem. In particular, mission goals were to determine the physical processes responsible for the generation and maintenance of the polynya, to study the hydrodynamic control of carbon cycling, and to investigate the sequestration of carbon. In addition, numerous biological and rate process measurements were made on organisms ranging from bacteria to whales (Deming et al. 2002). The incorporation of such information into ecosystem models can be used to assess the impact

of global warming on the North Water and perhaps, the entire arctic ecosystem. One of the biological goals was to establish and study connections between copepods and the microbial food web. Thus far, the role of omnivory in polynyas has not been rigorously investigated.

## 1.5 Thesis overview

In this thesis, the lipid compositions of three dominant copepod species, *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* CVI (female), are used to investigate diet and feeding strategy in the North Water Polynya. Chapters II and III deal largely with methodological issues; specifically, the testing of a lipid sample processing method well suited to polar research (II) and the development of a new lipid-based omnivory index (III). Chapters IV and V report detailed species-specific and spatial patterns in omnivory indices in copepods sampled throughout the polynya.

The processing of lipid samples in remote oceanic regions is often affected by the availability of equipment, chemicals, space and time. In light of these constraints, it is common practice to freeze zooplankton samples at sea and sort and extract them later ("freeze-sort"). However, the validity of this method has not been explicitly addressed in the literature. In Chapter II, the robustness of freeze-sort samples is tested by comparing resulting lipid class and fatty acid data to those obtained using more conventional methods, specifically, sorting copepods prior to freezing ("sort-freeze"). For the tests, two copepod species from different genera, *Calanus hyperboreus* CV and *Metridia longa*

CVI (female) were used. It was found that the freeze-sort method is not always appropriate and thus recommendations for obtaining more accurate data are made.

A central goal of this thesis is the development of lipid-based omnivory indices capable of demonstrating *direct* connections between copepods and the microbial food web. For the most part, individual lipid molecules (e.g. specific fatty acids, sterols and hydrocarbons) are used as biomarkers. However, it is suggested in this thesis that broad lipid class data can also yield important dietary information. In Chapter III, a new index for the estimation of copepod feeding strategy is developed and tested during a long-term incubation with *Calanus glacialis* CV. During chromatographic development in a non-polar solvent system, wax esters (WE) of the three copepod species split according to the degree of acyl lipid unsaturation. It is proposed that the ratio between the polyunsaturated peak and total WE represents an omnivory index, the unsaturation coefficient (UC). Copepods with high UC are probably feeding herbivorously, while low UC suggests omnivory. Changes in copepod lipids and UC during a 6-week incubation with bacterivorous dinoflagellates and diatoms support this contention. The use of bacterial biomarkers as potential omnivory indices is also investigated.

In Chapter IV, the omnivory indices introduced in Chapter III, including UC and bacterial fatty acids, are compared between *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* CVI (female) from several regions of the polynya. The utility of these indices at demonstrating connections between copepods and the microbial food web is

investigated, with a focus on species-specific differences. The indices are in general agreement with previously established but less direct approaches. The data are discussed in the context of different life and feeding strategies adopted by each copepod species and in terms of *in situ* food availability and composition.

As a result of the convergence of water with multiple origins, distinct regional microplankton assemblages characterize the North Water. Spatial gradients in primary productivity, abundances of specific phytoplankton groups and protists, and nutrient concentrations have been established (Klein et al. 2002; Lovejoy et al. 2002; Tremblay et al. 2002). In Chapter V, principal components analysis is used to investigate spatial patterns in the lipid compositions of *Calanus hyperboreus* CV, *C. glacialis* CV, *Metridia longa* CVI (female) sampled extensively throughout the polynya. It is proposed that spatial changes in the degree of copepod omnivory and proportions of bacterial markers observed are related to localized microbial conditions.

### 1.6 Co-authorship Statements

I am the first author on all research papers included in this thesis. I designed the experiments, planned the sampling programs, participated in data collection, performed the analyses and wrote the manuscripts. Drs. Don Deibel and Christopher C. Parrish are co-authors on all papers and contributed to the elaboration of ideas, provided practical advice, and made many helpful editorial comments on earlier drafts. Dr. Patricia A. Saunders helped greatly with experimental methodology and scientific discussions

concerning Chapter II. Sources of complementary data kindly shared by scientists involved in *The International North Water Polynya Study* are acknowledged in the text.

Publication and submission status:

Chapter II (Stevens, Deibel, Saunders, Parrish) has been submitted to *Marine Ecology Progress Series*

Chapter III (Stevens, Deibel, Parrish) has been accepted for publication in *Journal of Experimental Marine Biology and Ecology*

Chapter IV (Stevens, Deibel, Parrish) has been accepted for publication in *Marine Biology*

Chapter V (Stevens, Deibel, Parrish) has been submitted to *Deep Sea Research Part I*

## 1.7 References


Ackman RG, Tocher CS (1968) Marine phytoplankter fatty acids. J Fish Res Board Can 25:1603-1620

Atkinson A (1995) Omnivory and feeding selectivity in five copepod species during spring in the Bellingshausen Sea, Antarctica. ICES J Mar Sci 52:385-396

Atkinson A (1996) Subantarctic copepods in an oceanic, low chlorophyll environment: ciliate predation, food selectivity and impact on prey populations. Mar Ecol Prog Ser 130:85-96

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Riel LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257-263

Brett MT, Müller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshw Biol 38:483-499

Closs GP, Balcombe SR, Shirley MJ (1999) Generalist predators, interaction strength and food web stability. Adv Ecol Res 28:93-126

Cripps GC, Hill HJ (1998) Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. Deep-Sea Res I 45:1357-1381

Cushing DH (1989) A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. J Plankton Res 11:1-13

Deming JW, Fortier L, Fukuchi M (2002) The International North Water Polynya Study (NOW): a brief overview. Deep-Sea Res II 49:4887-4892

DeMott WR, Müller-Navarra D (1997) The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. Freshw Biol 38:649-664

Desvilettes Ch, Bourdier G, Breton JC, Combrouze Ph (1994) Fatty acids as organic markers for the study of trophic relationships in littoral cladoceran communities. J Plankton Res 16:643-659

Fagan WF (1997) Omnivory as a stabilizing feature of natural communities. Am Nat 150:554-567

Falk-Petersen S, Sargent JR, Tande KS (1987) Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol 8:115-120

Fenchel T (1988) Marine plankton food chains. Annu Rev Ecol Syst 19:19-38

Froneman PW, Pakhomov EA, Perissinotto R, McQuaid CD (1996) Role of microzooplankton in the diet and daily ration of Antarctic zooplankton species during austral summer. Mar Ecol Prog Ser 143:15-23

Gifford DJ (1991) The protozoan-metazoan trophic link in pelagic ecosystems. J Protozool 38:81-86

Gifford DJ, Fessenden LM, Garrahan PR, Martin E (1995) Grazing by microzooplankton and mesozooplankton in the high-latitude North Atlantic Ocean: spring *versus* summer dynamics. J Geophys Res 100:6665-6675

Gismervik I, Anderson T (1997) Prey switching by *Acartia clausi*: experimental evidence and implications of intraguild predation assessed by a model. Mar Ecol Prog Ser 157:247-259

Graeve M, Hagen W, Kattner G (1994a) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep-Sea Res I 41:915-924

Graeve M, Kattner G, Hagen W (1994b) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97-110

Greene CH (1988) Foraging tactics and prey-selection patterns of omnivorous and carnivorous calanoid copepods. Hydrobiologia 167/168:295-302

Harris RP (1996) Feeding ecology of *Calanus*. Ophelia 44:85-109

Hobson KA, Fisk A, Karnovsky N, Holst M, Gagnon J-M, Fortier M (2002) A stable isotope ($d^{13}C$, $d^{15}C$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep-Sea Res II 49:5131-5150

Hopkins TL (1987) Midwater food web in McMurdo Sound, Ross Sea, Antarctica. Mar Biol 96:93-106

Hopkins TL, Torres JJ (1989) Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep-Sea Res I 36:543-560

Hughes RN (1980) Optimal foraging theory in the marine context. Oceanogr Mar Biol Ann Rev 18:423-481

Huntley M (1988) Feeding biology of *Calanus*: a new perspective. Hydrobiologia 167/168:83-99

Jørgensen CB (1966) Biology of suspension feeding. Pergamon Press

Juhl AR, Ohman MD, Goericke R (1996) Astaxanthin in *Calanus pacificus*: Assessment of pigment-based measures of omnivory. Limnol Oceanogr 41:1198-1207

Kaneda T (1991) Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol Rev 55:288-302

Kattner G, Hirche HJ, Krause M (1989) Spatial variability in lipid composition of calanoid copepods from Fram Strait, the Arctic. Mar Biol 102:473-480

Kiørboe T, Saiz E, Viitasalo M (1996) Prey switching behaviour in the planktonic copepod *Acartia tonsa*. Mar Ecol Prog Ser 143:65-75

Klein B, LeBlanc B, Mei Z-P, Beret R, Michaud J, Mundy C-J, von Quillfeldt CH, Garneau M-È, Roy S, Gratton Y, Cochran JK, Bélanger S, Larouche P, Pakulski JD, Rivkin RB, Legendre L (2002) Phytoplankton biomass, production and potential export in the North Water. Deep-Sea Res II 49:4983-5002

Kleppel GS (1993) On the diets of calanoid copepods. Mar Ecol Prog Ser 99:183-195

Kondoh M (2003) Foraging adaptation and the relationship between food-web complexity and stability. Science 299:1388-1391

Landry MR (1981) Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. Mar Biol 65:77-82

Lee RF, Nevenzel JC, Paffenhöfer G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9:99-108

Lehman JT (1976) The filter feeder as an optimal forager, and the predicted shapes of feeding curves. Limnol Oceanogr 21:501-516

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204:65-77

Lovejoy C, Legendre L, Martineau M-J, Bâcle J, von Quillfeldt CH (2002) Distribution of phytoplankton and other protists in the North Water. Deep-Sea Res II 49:5027-5047

McCann K, Hastings A, Huxel GR (1998) Weak trophic interactions and the balance of nature. Nature 395:794-798

Meziane T, Bodineau L, Retiere C, Thoumelin G (1997) The use of lipid markers to define sources of organic matter in sediment and food web of the intertidal salt-marsh-flat ecosystem of Mont-Saint-Michel Bay, France. J Sea Res 38:47-58

Meziane T, Tsuchiya M (2000) Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar Ecol Prog Ser 200:49-57

Murdoch WW, Oaten A (1975) Predation and population stability. Adv Ecol Res 9:1-131

Nejstgaard JC, Gismervik I, Solberg PT (1997) Feeding and reproduction by *Calanus finmarchicus*, and microzooplankton grazing during mesocosm blooms of diatoms and the coccolithophore *Emiliania huxleyi*. Mar Ecol Prog Ser 147:197-217

Nejstgaard JC, Hygum BH, Naustvoll L-J, Båmstedt U (2001) Zooplankton growth, diet and reproductive success compared in simultaneous diatom- and flagellate-microzooplankton dominated plankton blooms. Mar Ecol Prog Ser 221:77-91

Oaten A, Murdoch WW (1975) Switching, functional response, and stability in predator-prey systems. Am Nat 109:299-318

Ohman MD, Runge JA (1994) Sustained fecundity when phytoplankton resources are in short supply: Omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. Limnol Oceanogr 39:21-36

Parrish CC, Yang Z, Lau A, Thompson RJ (1996) Lipid composition of *Yolida hyperborea* (Protobranchia), *Nephthys ciliata* (Nephthyidae) and *Artacama proboscidea* (Terebellidae) living at sub-zero temperatures. Comp Biochem Physiol 114B:59-67.

Pimm SL, Lawton JH (1977) Number of trophic levels in ecological communities. Nature 268:329-331

Pimm SL, Lawton JH (1978) On feeding on more than one trophic level. Nature 275:542-544

Polis GA, Strong DR (1996) Food web complexity and community dynamics. Am Nat 147:813-846

Pomeroy LR (1974) The ocean's food web, a changing paradigm. BioScience 24:499-504

Poulet SA (1983) Factors controlling utilization of non-algal diets by particle-grazing copepods. A review. Oceanol Acta 6:221-234

Pranal V, Fiala-Médioni A, Guezennec J (1996) Fatty acid characteristics in two symbiotic gastropods from a deep hydrothermal vent of the West Pacific. Mar Ecol Prog Ser 142:175-184

Richman S, Rogers JN (1969) Feeding of *Calanus helgolandicus* on synchronously growing populations of the marine diatom *Ditylum brightwellii*. Limnol Oceanogr 14;701-709

Roman MR, Rublee PA (1980) Containment effects in copepod grazing experiments: a plea to end the black box approach. Limnol Oceanogr 25:982-990

Runge JA (1988) Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. Hydrobiologia 167/168:61-71

Sargent JR, Eilersten HC, Falk-Petersen S, Taasen JP (1985) Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. Mar Biol 85:109-116

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleig MA (ed) Microbes in the Sea. Ellis Harwood, Chichester, p 119-138

Saunders PA, Deibel D, Stevens CJ, Rivkin RB, Lee S-H, Klein B (2003) Copepod herbivory rate in a large arctic polynya and its relationship to seasonal and spatial variation in copepod and phytoplankton biomass. Mar Ecol Prog Ser 261:183-199

Sherr EB, Sherr BF, Paffenhöfer G-A (1986) Phagotrophic protozoa as food for metazoans: a "missing" trophic link in marine pelagic food webs? Mar Microb Food Webs 1:61-80

Sherr E, Sherr B (1988) Role of microbes in pelagic food webs: a revised concept. Limnol Oceanogr 33:1225-1227

Sprules WG, Bowerman JE (1988) Omnivory and food chain length in zooplankton. Ecology 69:418-426

Sterner RW, Bajpai A, Adams T (1997) The enigma of food chain length: absence of theoretical evidence for dynamic constraints. Ecology 78:2258-2262

Stirling I (1997) The importance of polynyas, ice edges, and leads to marine mammals and birds. J Mar Syst 10:9-21

Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. J Plankton Res 12:891-908

Tremblay J-E, Gratton Y, Fauchot J, Price NM (2002) Climatic and oceanic forcing of new, net, and diatom production in the North Water. Deep-Sea Res II 49:4927-4946

Urban JL, McKenzie CH, Deibel D (1993) Nanoplankton found in fecal pellets of macrozooplankton in coastal Newfoundland waters. Bot Mar 36:267-281

Viso A-C, Marty J-C (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34:1521-1533

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128:219-240

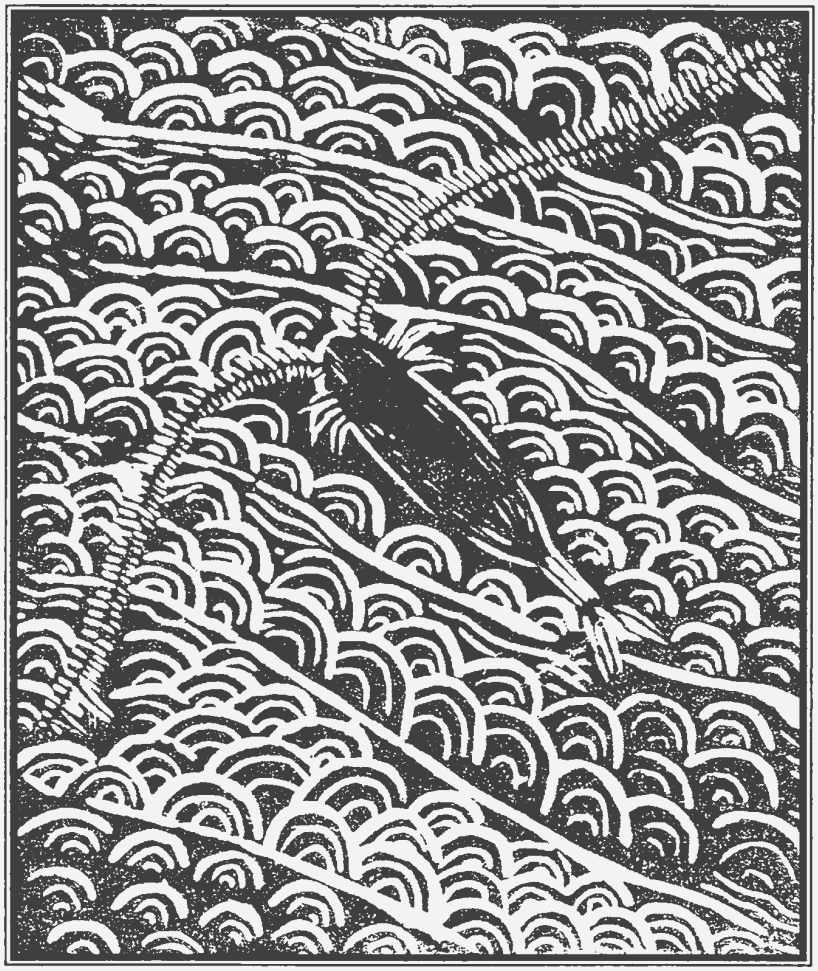
Volkman JK, Barrett SM, Blackburn SI, Mansour MP, Sikes EL, Gelin F (1998) Microalgal biomarkers: A review of recent research developments. Org Geochem 29:1163-1179.

Yager PL, Wallace DWR, Johnson KM, Smith WO, Minnett PJ, Deming JW (1995) The Northeast Water Polynya as an atmospheric $CO_2$ sink: a seasonal rectification hypothesis. J Geophys Res 100:4389-4398

Yang Z, Parrish CC, Helleur RJ (1996) Automated gas chromatographic method for neutral lipid carbon number profiles in marine samples. J Chromatogr Sci 34:556-568.

Zhukova NV, Kharlamenko VI, Svetashev VI, Rodionov IA (1992) Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. J Exp Mar Biol Ecol 162:253-263

# Chapter 2. Integrity of copepod lipid samples using individuals sorted from live *versus* frozen mixtures



## 2.1 Abstract

Lipid class and fatty acid data in samples of *Metridia longa* (females) and *Calanus hyperboreus* (CV) processed in two different ways were compared. The relatively quick "freeze-sort" method, in which copepod assemblages were frozen together at sea and sorted later, overestimated amounts of wax esters, triacylglycerols and total lipid (TL: sum of lipid classes) in *M. longa*, relative to conventionally processed "sort-freeze" samples (animals sorted live in the field). These increases in lipid in freeze-sort samples were likely due to leakage and contamination by adjacent *Calanus* spp. during processing. In *C. hyperboreus*, the two processing methods yielded comparable lipid class data, although acetone-mobile polar lipids (< 4% of TL) were overestimated by the freeze-sort method. Amounts of all fatty acid groups (odd-numbered and/or branched (OBFA), saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA)) were equivalent in sort-freeze and freeze-sort samples of *M. longa*. In *C. hyperboreus*, SFA, MUFA and PUFA levels did not differ with method, while proportions of OBFA were significantly higher in sort-freeze samples. The freeze-sort method, which saved considerable processing time in the field, provided accurate broad scale lipid data for *C. hyperboreus* and fatty acid data for *M. longa*, but was inappropriate for sensitive analyses of certain biomarkers in the former species and lipid classes in the latter. Recommendations are made to reduce interspecific lipid contamination during freeze-sort sample processing.

## 2.2 Introduction

Lipids are useful tools in the study of copepod ecology. Monitoring lipid levels and changes in composition over time has helped decipher copepod diapause and reproductive investment (Hagen and Schnack-Schiel 1996, Jónasdóttir 1999), buoyancy and seasonal ascent (Visser and Jónasdóttir 1999), and responses to *in situ* food availability (Lee 1974, Falk-Petersen et al. 1987). Lipids are also used as biomarkers to trace the ingestion of specific prey by copepods (Lee et al. 1971, Graeve et al. 1994a, Ederington et al. 1995), providing a more comprehensive understanding of their roles in food webs (Norrbin et al. 1990, Graeve et al. 1994b, Ward et al. 1996, Falk-Petersen et al. 1999).

When using copepod lipid levels as proxies of ecological processes, it is important to obtain accurate estimates. Storage conditions and sample handling (*i.e.*, how copepods are processed prior to storage/analysis) can both affect the accuracy of lipid measurements. Although the freezing process itself does not affect lipid composition, enzymatic hydrolysis occurs in samples stored at -15°C, resulting in accumulation of free fatty acids and phospholipid loss (Ohman 1996). To ensure lipid integrity, Ohman (1996) recommended that samples be stored below -70°C. Sample integrity can also be maintained at higher temperatures (*e.g.*, -20°C) if solvent (*e.g.*, chloroform) is added (Sasaki and Capuzzo 1984). The effect of different methods of sample processing on lipid measurement accuracy has not been rigorously addressed in the literature.

Many researchers sort live copepods in the field and freeze animals in chloroform (Falk-Petersen et al. 1999, Scott et al. 2000), on filters (Ederington et al. 1995) or in vials (Hagen and Schnack-Schiel 1996, Jónasdóttir 1999). This conventional processing method is referred to here as "sort-freeze" sampling. Where sorting is not logistically possible, researchers freeze batches of unsorted copepods together on filters, thawing and sorting them later (Ohman et al. 1989, Ward et al. 1996, Cotonnec et al. 2001); this protocol is hereafter referred to as "freeze-sort" sampling. This approach is appealing in that it requires less ship time, little equipment, and no chemicals.

The question is: can "freeze-sort" samples provide quantitative lipid data comparable to "sort-freeze" samples? Although to my knowledge this question has not been dealt with in the literature, it is assumed by many that damage to copepods during the processing of "freeze-sort" samples will yield inaccurate estimates of lipid due to leakage and contamination by adjacent individuals (G. Kattner, pers. comm.). In this chapter, the integrity of the "freeze-sort" method was tested, in terms of total lipid, lipid class and fatty acid data, using samples of *Metridia longa* (females) and *Calanus hyperboreus* (CV).

## 2.3 Materials and Methods

Copepods were collected at six stations in the North Water Polynya (Northern Baffin Bay) using a messenger-activated closing net system equipped with 200 μm mesh

nets and partially closed codends. Discrete depth strata were sampled with vertical tows between 4 September and 1 October 1999 (Table 2.1).

### *2.3.1 Sort-freeze samples*

Triplicate samples of *Metridia longa* females (Stations 1, 2, 3) and *Calanus hyperboreus* CV (Stations 4, 5, 6) were picked out of the net tow catches using the blunt end of a pipette, a dissecting microscope, and a 10 ml sorting cell. Sorted copepods were placed in specimen cups filled with ~50 ml filtered seawater (0.2 μm) and kept on ice. After a sufficient number was picked (Table 2.1), the contents of the cups were filtered onto combusted 25 mm GF/C filters and then folded, quick-frozen on an aluminum block (pre-cooled to -80°C), placed in combusted foil envelopes and stored at -80°C.

### *2.3.2 Freeze-sort samples*

At the same stations, unsorted assemblages of ~100-300 copepods were gently filtered onto combusted 47 mm GF/C filters, quick-frozen on the aluminum block and stored in individual Petri dishes at -80°C. When the fieldwork was completed, samples were packed with dry ice (along with sort-freeze samples) and sent to the laboratory (Ocean Sciences Centre, NL) where they were stored at -70°C until analysis. Filters were placed on a chilled stage, consisting of the smaller half of a large glass Petri dish nested in the larger half, which was filled with ice. Using fine probes and forceps, copepods were gently removed from the filters and spread around the stage for identification. Picking was terminated when the animals were almost entirely thawed (*ca.* 10-25 minutes

after removal from the freezer), because at this point they may start to lose lipid from the anal pore (Ohman 1996). In general, copepods were in very good condition: they appeared intact and were easily removed from the filters. Although lipid was lost via the anal pore in some individuals, this loss was minor (<5% of total, rough visual estimate). Loss of this magnitude was noted in about 10% of *Metridia longa* and 37% of *Calanus hyperboreus* individuals. If copepods were leaking lipid through the body wall, or if a relatively large amount appeared to have been lost through the anal pore (>5%), they were discarded. Sorted groups of copepods (Table 2.1) were added to test tubes containing 2 ml of chloroform (frozen sort-freeze filters also), flushed with $N_2$, sealed, and stored at -20°C until analysis.

### *2.3.3 Lipid Analysis*

Lipids were extracted following a modified version of Folch et al. (1957). Samples were ground, sonicated (4 min) and centrifuged (125 x g) four times in a 8:4:3 chloroform:methanol:water mixture, and the organic layers removed and pooled. To separate and quantify lipid classes, samples were manually spotted on silica-coated Chromarods (SIII) and passed through the flame-ionization detector of an Iatroscan MK V. Rod development was done following Parrish (1987). Total lipid was calculated as the sum of all lipid classes detected. This system does not separate wax and steryl esters. However, preliminary GC analysis indicates that samples of *Calanus hyperboreus* from the same region contain many wax esters but no steryl esters (Kehoe 2003). Wax ester (WE) isolated from *C. hyperboreus*, following the protocol of Ohman (1997), was used

as a calibrant for wax esters. Commercial standards were used for all other lipid classes. Ohman (1997) found that WE purified from *C. pacificus* CV was a suitable calibrant for females of this species, and for CVs and females of *Metridia pacifica*, *Eucalanus californicus* and *Rhincalanus nasutus*. Fatty acids were quantified as methyl esters using a Varian Model 3400 gas chromatograph, following total lipid derivatization of samples with $BF_3$-methanol (85°C, 1 h). Methyl esters were analysed on an Omegawax column following Budge and Parrish (1998). Tricosanoic acid (23:0; absent in copepods) was used as an internal standard, at a concentration of ~10% total fatty acids. Fatty acid peaks were identified using commercial standards as references.

### *2.3.4 Statistical Analysis*

To test for differences in lipid levels between sort-freeze and freeze-sort samples of *Metridia longa* and *Calanus hyperboreus* (tested separately), a randomized block ANOVA was used with method as the main effect and stations as blocks. In accordance with this design, between-station differences were not of interest and no interaction term was tested. Table 2.3 lists all the response variables tested for differences in method. A rejection criterion of $\alpha = 0.05$ was used to determine statistical significance.

## 2.4 Results and Discussion

Across all samples, wax esters (WE) were the main storage lipid in both *Metridia longa* and *Calanus hyperboreus* (71 - 91% of total lipid (TL)), with triacylglycerols (TG), phospholipids (PL) and acetone-mobile polar lipids (AMPL) present in smaller amounts

(< 15% of TL, Figure 2.1). Sterols, alcohols, hydrocarbons and free-fatty acids were present in very small quantities in both copepods (< 2% of TL, data not shown). In *M. longa*, WE and TG levels were significantly higher in freeze-sort samples, while AMPL and PL were equivalent in the two types of samples (Figure 2.1, Table 2.2). With the exception of AMPL levels, which were significantly higher in freeze-sort samples of *C. hyperboreus*, the two methods yielded comparable amounts of all other lipid classes (WE, TG, PL) tested for this species. In *C. hyperboreus*, there was no difference in TL between sort-freeze and freeze-sort samples, while in *M. longa*, TL was significantly higher in freeze-sort samples. As expected, TL was much higher (1 order of magnitude) for *C. hyperboreus* than for *M. longa*; the former is larger and stores more lipid per unit body mass (Lee and Hirota 1973).

Assuming for discussion that the sort-freeze method is more accurate than the freeze-sort method, any discrepancies between the two can be attributed to flaws in the latter. This assumption is probably sound given that many precautions against possible lipid loss and contamination are taken during sort-freeze sampling. The results therefore indicate that the freeze-sort method overestimated amounts of major lipid classes (WE by 14%, TG by 36%) in *Metridia longa*, and a minor lipid class (AMPL by 37%) in *Calanus hyperboreus*. In *M. longa*, the increased lipid (WE, TG) in freeze-sort samples was probably the result of contamination by neighboring copepods on the filters that may have leaked lipid from their anal pores during thawing and sorting. Most of the adjacent copepods were late stages of *Calanus* spp. (author's pers. obs.) that store enormous

amounts of WE (Sargent and Henderson 1986) and due to their large size and high lipid content, can also contain moderate absolute amounts of TG (Figure 2.1). Although it is suggested that these copepods leaked both WE and TG, the higher TL levels (by 15%) in freeze-sort samples of *M. longa* were probably due to WE contamination alone.

In the case of *Calanus hyperboreus*, higher levels of AMPL (includes chlorophyll) in freeze-sort samples may have arisen from the adherence of phytoplankton to the animals: clumps of cells were often seen on filters during sorting. If phytoplankton caused AMPL contamination in freeze-sort samples, rinsing animals after sorting may solve the problem. The estimates of TG and PL in *C. hyperboreus* were highly variable (coefficients of variation (cv) for TG: 35 - 173%; cv for PL: 5 - 94%). Because WE accounts for almost all the lipid in *C. hyperboreus* samples, less abundant classes (*i.e.*, TG, PL) in small aliquots applied to Chromarods approach detection limits and the resulting peaks are flattened and asymmetrical. This lowers measurement precision since it becomes difficult to distinguish these peaks from baseline noise. Alternatively, TG levels (and possibly PL) in *Calanus* spp. may be inherently variable, reflecting differing nutritional states of individual copepods in populations exposed to patchy food resources *in situ*. Hakanson (1984) found that TG content in *C. pacificus* responded rapidly (compared to WE) to changes in food concentration and starvation.

In both copepods, monounsaturated (MUFA, one double bond) and polyunsaturated fatty acids (PUFA, 2 or more double bonds) were the major fatty acid

groups (30 - 59% of total fatty acids, Figure 2.2). Odd-numbered and/or branched fatty acids (OBFA) and saturates (SFA, no double bonds) made up the rest of the total in both copepods (1 - 9% of total fatty acids). Except for OBFA levels in *Calanus hyperboreus*, which were significantly higher (by 30%) in sort-freeze samples, there were no differences in all other fatty acid groups tested, for either species (Figure 2.2, Table 2.3). Concentrations of most OBFA were elevated in sort-freeze samples of *C. hyperboreus* at all stations, relative to freeze-sort samples (Table 2.2). In fact, many OBFA present in small quantities in sort-freeze samples were not detected in the freeze-sort counterparts (Table 2.2, Stations 4 & 5). Perhaps this is because fewer copepods, and hence less lipid, were included in most freeze-sort samples (Table 2.1).

In both species, total fatty acids (TFA) were equivalent in sort-freeze and freeze-sort samples (Figure 2.2, Table 2.3). Although contamination of *Metridia longa* with WE probably occurred during freeze-sort sample processing, the fatty acid data do not reflect this. On average, copepod wax ester molecules are only 50% fatty acid by mass (compared to ~66% for PL (2 fatty acids + glycerol + phosphoric acid) and ~90% for TG (3 fatty acids + glycerol)), as they are composed of a fatty acid esterified to a fatty alcohol of similar carbon number (Sargent and Henderson 1986). Therefore, fatty acid contamination by WE will be half that (7%) of total lipid contamination by WE (14%), which was usually within the measurement error (cv of TFA estimates: 1 - 31%; mean=11%; n=6).

For the most part, the freeze-sort method yielded lipid data comparable to the more conventional sort-freeze method for *Calanus hyperboreus* CV. Therefore, it is suggested that the former method can provide accurate broad scale lipid data (*e.g.*, WE, TL, major fatty acid groups) for this species, but may sometimes be inappropriate for sensitive analyses of certain lipid classes (*e.g.*, AMPL) and biomarkers (*e.g.*, OBFA). For *Metridia longa* females, it appears that freeze-sort fatty acid data were fairly robust; however, reliable lipid class data were not obtained using this method. In cases where large, lipid-rich copepods (*e.g.*, *Calanus* spp.) are frozen together with smaller ones (*e.g.*, *Metridia* spp. and smaller), levels of contamination in the latter will probably be significant. This is especially true if the larger copepods have a greater tendency to leak, as was the case with *C. hyperboreus* (37% of individuals lost ~5% of TL *versus* 10% in *M. longa*). Leakage of this magnitude by *C. hyperboreus* was certainly within the measurement error (cv of TL estimates: 10 - 52%; mean=23%; n=6) suggesting that appreciable intraspecific contamination did not occur.

The robustness of the freeze-sort technique could be improved by: (1) optimizing the amount of lipid per sample (i.e., including as many animals as possible within analytical limits, which may improve quantitation of minor components like OBFA and TG), (2) rinsing animals after sorting to remove adhering phytoplankton, and (3) preparing the samples in ways that minimize interspecific contamination. In the least, this could be achieved by sampling fewer copepods per filter. Instead of overloading one filter with many animals, several sparse filters could be collected per station. This would

also ensure sufficient numbers of individual species and developmental stages of interest. Also, some pre-processing could be performed by roughly separating species into size classes by sieving.

## 2.5 Acknowledgements

The author would like to thank the officers, crew and captain of the *N.G.C.C. Pierre Radisson*, as well as M. Fortier, N. Kobayashi, M. Ringuette, M. Sampei, P. Sirois and K. Takahashi for assistance in the field. Funding for this project was provided by an NSERC grant awarded to D. Deibel, R. Rivkin and M. Anderson, the NSERC Discovery Grant of D. Deibel, and a Northern Scientific Training Program grant awarded to C. Stevens.

## 2.6 References


Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29:1547-1559

Cotonnec G, Brunet C, Sautour B, Thoumelin G (2001) Nutritive value and selection of food particles by copepods during a spring bloom of *Phaeocystis* sp. in the English Channel, as determined by pigment and fatty acid analyses. J Plankton Res 23:693-703

Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limnol Oceanogr 40:860-867

Falk-Petersen S, Sargent JR, Tande KS (1987) Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol 8:115-120

Falk-Petersen S, Sargent JR, Lønne OJ, Timofeev S (1999) Functional biodiversity of lipids in Antarctic zooplankton: *Calanoides acutus*, *Calanus propinquus*, *Thysanoessa macrura* and *Euphausia crystallorophias*. Polar Biol 21:37-47

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509

Graeve M, Kattner G, Hagen W (1994a) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97-110

Graeve M, Hagen W, Kattner G (1994b) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep-Sea Res I 41:915-924

Hagen W, Schnack-Schiel SB (1996) Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction? Deep-Sea Res I 43:139-158

Hakanson JL (1984) The long and short term feeding condition in field-caught *Calanus pacificus*, as determined from the lipid content. Limnol Oceanogr 29:794-804

Jónasdóttir SH (1999) Lipid content of *Calanus finmarchicus* during overwintering in the Faroe-Shetland Channel. Fish Oceanogr 8:61-72

Kehoe JJ (2003) Total marine lipid profiling by short column gas chromatography. MS thesis, Memorial University of Newfoundland, St. John's

Lee RF, Nevenzel JC, Paffenhöfer G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9:99-108

Lee RF, Hirota J (1973) Wax esters in tropical zooplankton and nekton and the geographical distribution of wax esters in marine copepods. Limnol Oceanogr 18:227-239

Lee RF (1974) Lipid composition of the copepod *Calanus hyperboreus* from the Arctic ocean. Changes with depth and season. Mar Biol 26:313-318

Norrbin MF, Olsen R-E, Tande KS (1990) Seasonal variation in lipid class and fatty acid composition of two small copepods in Balsfjorden, northern Norway. Mar Biol 105:205-211

Ohman MD, Bradford JM, Jillett JB (1989) Seasonal growth and lipid storage of the circumglobal, subantarctic copepod, *Neocalanus tonsus* (Brady). Deep-Sea Res I 36:1309-1326

Ohman MD (1996) Freezing and storage of copepod samples for the analysis of lipids. Mar Ecol Prog Ser 130:295-298

Ohman MD (1997) On the determination of zooplankton lipid content and the occurrence of gelatinous copepods. J Plankton Res 19:1235-1250

Parrish CC (1987) Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can J Fish Aquat Sci 44:722-731

Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) The biological chemistry of marine copepods. Clarendon Press, Oxford, pp 59-108

Sasaki GC, Capuzzo JM (1984) Degradation of *Artemia* lipids under storage. Comp Biochem Physiol 78B:525-531

Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR (2000) Lipids and life strategies of *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* in late autumn, Kongsfjorden, Svalbard. Polar Biol 23:510-516

Visser AW, Jónasdóttir SH (1999) Lipids, buoyancy and the seasonal vertical migration of *Calanus finmarchicus*. Fish Oceanogr 8:100-106

Ward P, Shreeve RS, Cripps GC (1996) *Rhincalanus gigas* and *Calanus simillimus*: lipid storage patterns of two species of copepod in the seasonally ice-free zone of the Southern Ocean. J Plankton Res 18:1439-1454

## 2.7 Tables

Table 2.1. Sampling details and description of copepod lipid samples (*Metridia longa* females and *Calanus hyperboreus* CV) where S-F represents sort-freeze samples and F-S, freeze-sort samples (samples taken in triplicate except for *M. longa* S-F samples at Station 2).

| Station | Date (1999) | Description | Copepods per sample | Location | Depth stratum sampled (m) |
|---|---|---|---|---|---|
| 1 | 04 Sept | *M. longa* | S-F: (25, 24, 24)<br>F-S: (9, 8, 8) | 78°18' N<br>74°27' W | 250 - 60 |
| 2 | 07 Sept | *M. longa* | S-F: (23, 20)<br>F-S: (10, 10, 10) | 76°17' N<br>71°55' W | 250 - 60 |
| 3 | 30 Sept | *M. longa* | S-F: (21, 20, 20)<br>F-S: (8, 7, 8) | 75°15' N<br>76°04' W | 75 - 0 |
| 4 | 11 Sept | *C. hyperboreus* | S-F: (6, 5, 5)<br>F-S: (3, 2, 2) | 78°22' N<br>74°43' W | 250 - 50 |
| 5 | 25 Sept | *C. hyperboreus* | S-F: (4, 3, 3)<br>F-S: (2, 2, 2) | 77°48' N<br>75°30' W | 100 - 0 |
| 6 | 01 Oct | *C. hyperboreus* | S-F: (4, 4, 4)<br>F-S: (4, 4, 3) | 75°34' N<br>70°46' W | 250 - 75 |

Table 2.2. Absolute amounts of all odd-numbered and or branched fatty acids detected in sort-freeze (S-F) and freeze-sort (F-S) samples of *Calanus hyperboreus* CV (errors are 1 standard deviation; nd=not detected).

| Fatty acid[a] | Station 4 | | Station 5 | | Station 6 | |
|---|---|---|---|---|---|---|
| (µg copepod-$^{1}$) | S-F | F-S | S-F | F-S | S-F | F-S |
| i-15:0 | 0.80 ± 0.17 | 0.95 ± 0.05 | 0.56 ± 0.27 | 0.37 ± 0.33 | 0.87 ± 0.08 | 0.41 ± 0.70 |
| ai-15:0 | 0.21 ± 0.36 | nd | 0.16 ± 0.14 | nd | 0.81 ± 0.24 | 0.67 ± 0.60 |
| 15:0 | 0.61 ± 0.09 | 0.43 ± 0.20 | 0.32 ± 0.07 | 0.16 ± 0.16 | 1.52 ± 0.60 | 1.31 ± 0.46 |
| 15:1 | 0.82 ± 0.07 | 0.30 ± 0.31 | 0.42 ± 0.10 | nd | 0.99 ± 0.44 | 0.77 ± 0.37 |
| i-16:0 | 0.17 ± 0.29 | nd | nd | nd | 0.13 ± 0.22 | 0.13 ± 0.22 |
| i-17:0 | nd | nd | 0.11 ± 0.10 | nd | 1.28 ± 0.20 | 1.23 ± 0.34 |
| ai-17:0 | 2.30 ± 0.31 | 1.53 ± 0.79 | 1.48 ± 0.37 | 1.32 ± 0.21 | 1.45 ± 0.57 | 1.32 ± 0.23 |
| 17:1 | 1.10 ± 0.78 | 0.23 ± 0.26 | 0.28 ± 0.16 | 0.22 ± 0.02 | 0.24 ± 0.41 | 0.23 ± 0.21 |
| TOTAL | 6.00 ± 0.35 | 3.44 ± 1.46 | 3.31 ± 0.62 | 2.15 ± 0.83 | 7.29 ± 1.77 | 6.05 ± 2.34 |

[a]small quantities (<0.1 µg copepod$^{-1}$) of 17:0 were detected in fewer than half of the samples

Table 2.3. Results of statistical analyses, testing numerous response variables in *Metridia longa* females and *Calamus hyperboreus* CV for significant differences due to method (ns=not significant).

| RESPONSE VARIABLE | *Metridia longa* METHOD | | *Calamus hyperboreus* METHOD | |
|---|---|---|---|---|
| | F ratio | p-value | F ratio | p-value |
| Wax esters | $F_{1,13}$=6.70 | 0.02 | $F_{1,14}$=0.06 | ns |
| Triacylglycerols | $F_{1,13}$=5.86 | 0.03 | $F_{1,14}$=2.14 | ns |
| Acetone-mobile polar lipids | $F_{1,13}$=0.40 | ns | $F_{1,14}$=6.73 | 0.02 |
| Phospholipids | $F_{1,13}$=3.46 | ns | $F_{1,14}$=3.71 | ns |
| Total lipid | $F_{1,13}$=9.09 | 0.01 | $F_{1,14}$=0.16 | ns |
| | | | | |
| Odd and/or branched fatty acids | $F_{1,12}$=0.17 | ns | $F_{1,14}$=6.70 | 0.02 |
| Saturated fatty acids | $F_{1,12}$=2.70 | ns | $F_{1,14}$=0.18 | ns |
| Monounsaturated fatty acids | $F_{1,12}$=3.76 | ns | $F_{1,14}$=0.07 | ns |
| Polyunsaturated fatty acids | $F_{1,12}$=0.09 | ns | $F_{1,14}$=1.82 | ns |
| Total fatty acids | $F_{1,12}$=1.53 | ns | $F_{1,14}$=0.73 | ns |

## 2.8 Figures

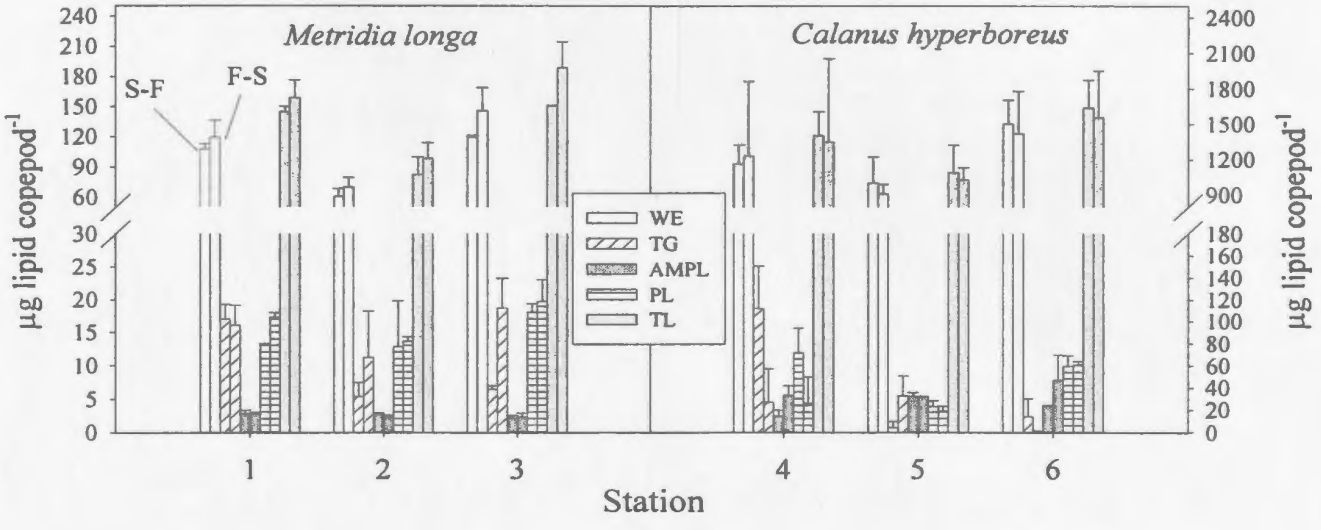



Figure 2.1. Mean amounts (µg lipid copepod$^{-1}$) of the principal lipid classes (wax esters (WE), triacylglycerols (TG), acetone-mobile polar lipids (AMPL), phospholipids (PL)) and total lipid (TL, the sum of all lipid classes including those < 2% TL: hydrocarbons, free-fatty acids, alcohols, sterols, data not shown) in *Metridia longa* females and *Calanus hyperboreus* CV. At each station, the first bar of each lipid class (and TL) represents sort-freeze samples (S-F) and the second bar, freeze-sort samples (F-S). Error bars are 1 standard deviation.

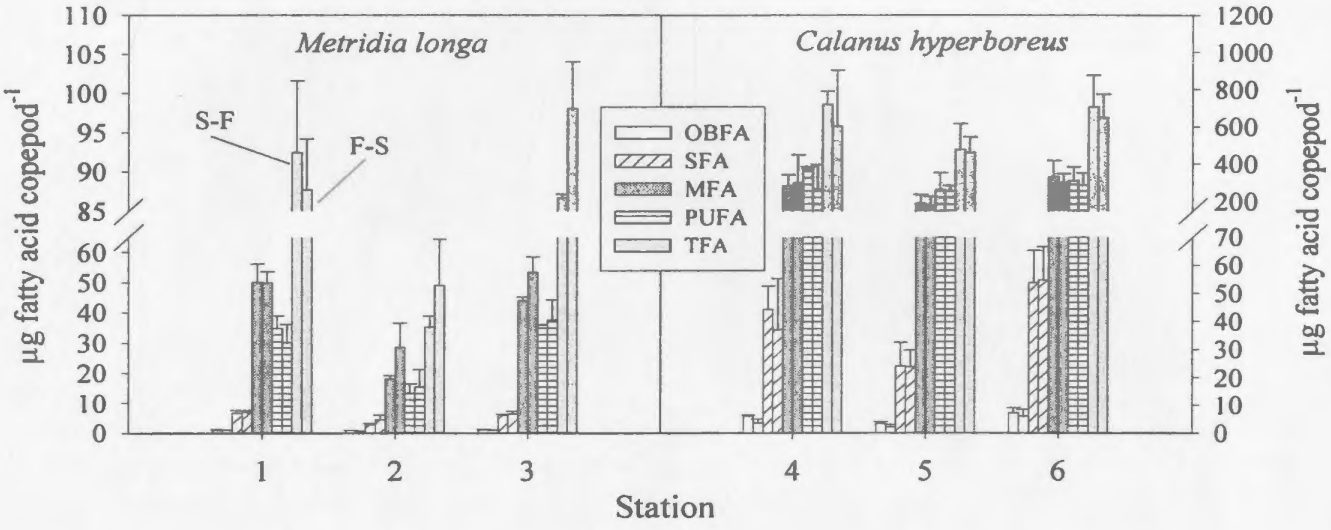
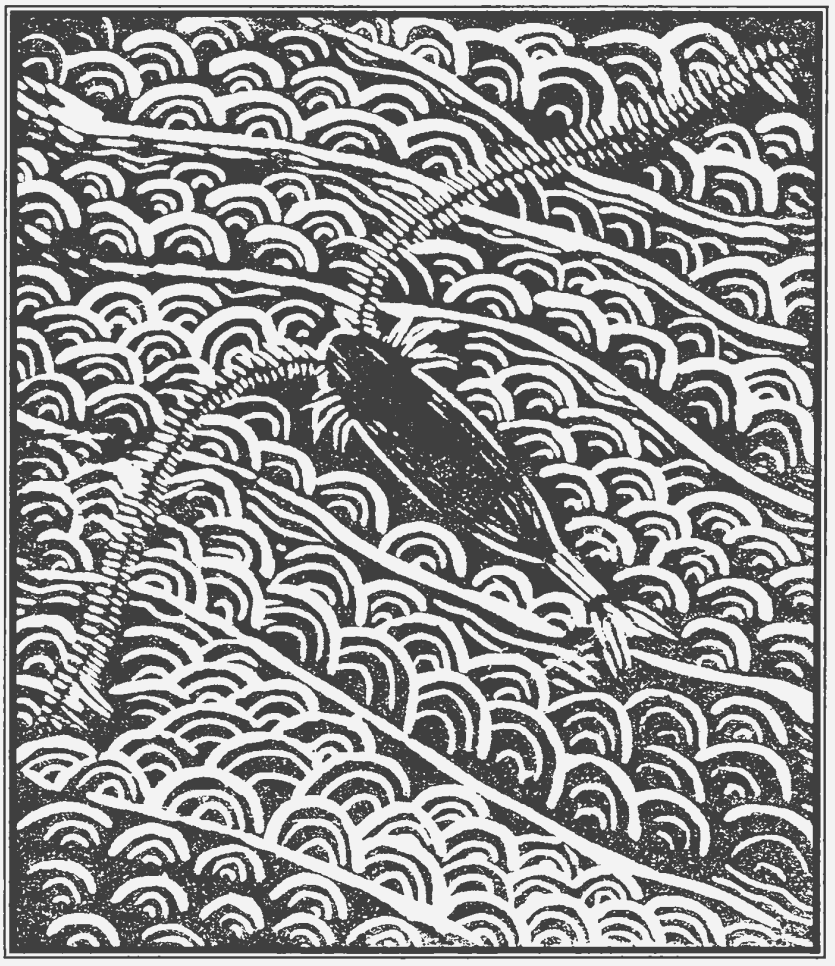



Figure 2.2. Mean amounts (µg fatty acid copepod$^{-1}$) of fatty acid groups (odd and/or branched (OBFA), saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA)) and total fatty acids (TFA, the sum of all fatty acids) in *Metridia longa* females and *Calanus hyperboreus* CV. Organization of bars as in Figure 2.1.

# Chapter 3. Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with *Calanus glacialis* Jaschnov



## 3.1 Abstract

The incorporation of fatty acid biomarkers into *Calanus glacialis* Jaschnov (CV) was traced during a long-term incubation experiment using bacterivorous dinoflagellates and diatoms as food. Copepods fed *Oxyrrhis marina* Dujardin during a 3-week acclimation period developed an omnivorous lipid composition, relative to wild-captured copepods, characterized by significant losses of polyunsaturated fatty acids (PUFA) and diatom fatty acids (16:4(n-1), 20:5(n-3)) and increases in saturated fatty acids and 18:1(n-7). Levels of a wax ester-based omnivory index (unsaturation coefficient, UC), verified by gas chromatography, also decreased in response to the relatively PUFA-poor dinoflagellate. After half of the copepods were switched to a diet comprised of the diatom *Thalassiosira hispida* Syvertsen (PUFA-rich), the data showed reversal to a more herbivorous lipid composition (increases in UC and relative amounts of PUFA and diatom fatty acids). Therefore, it is suggested that UC, derived from routine thin-layer chromatography analysis (Iatroscan), can quickly determine *in situ* feeding strategies (i.e., degree of omnivory) of wax ester-storing copepods. None of the eight odd and/or branched bacterial fatty acids (OBFA) initially detected in *C. glacialis* increased in response to a diet of *O. marina*, which was rich in these compounds (mainly i-15:0 and ai-15:0). Lack of transfer of these and other fatty acids (e.g., 22:6(n-3)) could be related to the physiological state of the copepods (early diapause). The bacterial fatty acid 18:1(n-7) may be more useful in inferring connections between *Calanus* spp. and the microbial food web than are odd and/or branched chains.

## 3.2 Introduction

Lipid biomarkers are very useful in the elucidation of trophic relationships among marine plankton. Lee et al. (1971) were the first to demonstrate the conservative transfer of ingested diatom fatty acids (16:1(n-7), 20:5(n-3)) into the wax esters of the boreal pelagic copepod *Calanus helgolandicus* during controlled laboratory experiments. Since then, fatty acids characteristic of diatoms (16:1(n-7)), dinoflagellates (18:4(n-3), 22:6(n-3)), prymnesiophytes (18:5(n-3)), and metazoans (18:1(n-9)) have been used to infer *in situ* ingestion of these prey by copepods in a number of marine systems (Sargent et al. 1985; Kattner et al. 1989; Cripps and Hill 1998; Falk-Petersen et al. 1999).

It has been suggested that elevated levels of bacterial fatty acids (odd and/or branched; (n-7) and (n-9) monounsaturates) in cladocerans (Desvilettes et al. 1994), littoral crabs and gastropods (Meziane and Tsuchiya 2000), deep-sea gastropods (Pranal et al. 1996), bivalves (Ben-Mlih et al. 1992; Zhukova et al. 1992) and polychaetes (Meziane et al. 1997) indicate *in situ* feeding on (or assimilation of) microbial material (e.g., bacterivorous ciliates, sediment, free bacteria, symbiotic bacterial synthate). Although *in vitro* transfer of bacterial fatty acids (ai-15:0, 17:0) between bacteria, bacterivorous ciliates, and *Acartia tonsa* has been demonstrated (Edderington et al. 1995), their contribution to copepod lipids *in situ*, especially to dominant oceanic species (e.g., *Calanus* spp.), has not been determined. Under certain conditions, *Calanus* spp. form important links between the microbial food web and higher trophic levels (Runge

and de Lafontaine 1996) and often prefer protozoans to phytoplankton in incubation experiments (Levinsen et al. 2000).

Although lipid biomarkers provide true *in situ* diet histories (unlike incubations), the data are qualitative and cannot furnish ingestion rates. Information on the time scales over which amounts and proportions of lipid vary in response to biological variables (e.g., diet, prey concentration, prey physiological state, copepod feeding history) is required to determine quantitative energy flow between predator and prey. At present, very little information exists on the *in vivo* turnover of lipid biomarkers in copepods on an ecologically relevant time scale. In an important series of experiments (Graeve et al. 1994), copepods caught over a broad geographical area (West Spitsbergen to East Greenland Shelf), acclimated to feeding on either diatom- or dinoflagellate-dominated seston, were switched to the opposite prey type. Six weeks were required for the complete replacement of the "wild" fatty acid signal with that of a monoalgal culture for *Calanus hyperboreus* (CV), *C. finmarchicus* (CV) and *C. glacialis* (CVI females). These results provide a time frame that integrates *in situ* "snapshot" data and illustrates the response times of copepod lipids to changes in seston composition. Similar studies, using prey rich in bacterial fatty acids, could reveal the *in vivo* behavior and usefulness of these biomarkers in demonstrating connections between copepods and the microbial food web.

Most lipid biomarkers, including fatty acids, are resolved during gas chromatography where molecules are separated for individual quantification. On the

other hand, thin-layer chromatography is used to quantify broad, ecologically relevant lipid classes indicative of energy storage, membrane allocation, pollution and sample degradation (reviewed in Parrish 2000). Rarely are broad lipid classes, even those highly influenced by ingested prey (e.g., triacylglycerols), used as dietary biomarkers. However, there is evidence that peak splitting, routinely observed during the analysis of certain lipid classes (free fatty acids, triacylglycerols, wax and steryl esters), represents a partial separation of esterified fatty acids on the basis of unsaturation (Oshima et al. 1987; Parrish et al. 1992). Because microplankton prey groups (e.g., dinoflagellates, diatoms, ciliates) have distinctive lipid compositions (Claustre et al. 1989; Viso and Marty 1993; Harvey et al. 1997), including somewhat predictable degrees of fatty acid unsaturation (e.g., higher in phytoplankton than in bacterivorous microzooplankton), ratios between split peaks may be useful omnivory indices.

During a long-term incubation, changes in the lipids of *Calanus glacialis* in response to ingestion of prey with a high proportion of bacterial fatty acids were monitored. This experiment included an acclimation period wherein all copepods were fed the bacterivorous dinoflagellate *Oxyrrhis marina* (CCAP 1133/4). After this period, half the copepods were switched to a diatom diet. By studying the incorporation and dampening of lipid signals over an ecological time scale of 6 weeks, it was determined whether bacterial, and other diagnostic fatty acids, could be used to track ingestion of bacterivorous microzooplankton by *C. glacialis*. In addition, I introduce and provide

experimental evidence for a new omnivory index that makes use of the natural splitting of copepod wax esters during thin-layer chromatography analysis.

## 3.3 Materials and Methods

### *3.3.1 Cultures*

The diatom *Thalassiosira hispida* (Table 3.1) was isolated from water collected near the Ocean Sciences Centre (Logy Bay, Newfoundland) and grown in f/2 medium (Guillard and Ryther 1962) at ~4°C, on a light:dark cycle of 18:6 h. Diatoms were grown in semi-continuous xenic culture in 4-l Pyrex flasks under constant aeration and were harvested for experiments during late exponential phase. The bacterivorous dinoflagellate *Oxyrrhis marina* (CCAP 1133/4) was grown in an artificial seawater + barley medium (per litre: 3.75 ml extra salt stock, 2.5 ml vitamin stock, 25 ml soil extract, 0.5 g tricine, 40 barley grains; see CCAP website) and maintained in 1-2 l Pyrex flasks in dim light at ~17°C. To harvest *O. marina*, barley fragments were removed from exponential phase cultures with 40 μm Nitex mesh. To separate bacteria from dinoflagellates, the culture was spun in a refrigerated centrifuge (Sorvall RC-513) at 30 x g for 30 minutes, the supernatant discarded, and the pellet twice rinsed and re-spun in autoclaved filtered seawater (0.35 μm). Using this method, it was found that few dinoflagellates remained in the supernatant (< 10%), cells appeared healthy (i.e., normal motility and behavior), and ~80% of the bacteria was removed (data not shown). To obtain carbon measurements of cultures, 30-50 ml were collected on combusted 25 mm GF/F filters, dried (50-60°C),

rolled in tin foil and run on a Perkin Elmer 2400 elemental analyzer (calibrated with acetanilide at a replicate precision of 96-99%).

### 3.3.2 *Incubation experiment*

Copepods were collected in vertical net tows (110 μm mesh) between 225 m and the surface on 21 August 2001 at one station in Conception Bay, Newfoundland (47°32'N, 53°0.8'W). Using a dissecting microscope and the blunt end of a pipette, ~500 *Calanus glacialis* CV were picked out of the net tow catches, placed in aerated filtered seawater (0.35 μm), and kept in dim light at 2°C until the experiment began. Six 2-l Pyrex beakers containing 80 copepods each were set up. Each was lightly aerated with an air stone and the top covered with aluminum foil. For the first 3 weeks (7-30 Sept), animals in all six beakers were fed *Oxyrrhis marina* (acclimation period); afterward (30 Sept-19 Oct), half were switched to *Thalassiosira hispida* (treatment) and half were kept on *O. marina* (control). Prey was provided at a concentration of ~400-500 μg C $l^{-1}$ $day^{-1}$ (Table 3.1).

Initially, and every 3-4 days thereafter, prey densities were measured under 'initial' and 'final' conditions (see below) for the determination of copepod ingestion rates (Frost 1972; Marin et al. 1986). To measure prey densities, beaker contents were mixed by gentle stirring, 1-2 ml removed, and cell numbers determined on a Coulter Multisizer II. At the end of each measurement interval, 'final' cell samples were taken, old incubation water was gently siphoned off and replaced with fresh, aerated (at least 12

h) filtered seawater, new prey was provided and cell numbers determined ('initial'), and dead copepods counted and removed. Two controls (prey only) per prey type were also set up to estimate culture growth during each measurement interval.

Copepod lipid samples were taken nine times during the experiment: at t=0 and every 3-4 (day 3-10) or 7 days (day 13-42) thereafter. Before the addition of new prey, 3 copepods from each beaker were removed by wide-bore pipette, filtered onto combusted 25 mm GF/C filters, placed in 2 ml chloroform and stored at -20°C under $N_2$ atmosphere. On the last day of the experiment (day 42), the number of copepods removed for lipid analysis was often > 3. Each time fresh culture was supplied to copepods, a sample was taken for lipid analysis: 32-50 ml were collected on combusted 25 mm GF/F filters and stored as above.

*3.3.3 Isolation and fractionation of wax ester*

Copepods were collected at one station in the North Water Polynya (Northern Baffin Bay: 78°20'N, 73°20'W; NOW Station 6) on 13 Sept 1999 using vertical hauls (200 μm mesh nets) between 150 and 0 m. Unsorted assemblages were filtered onto combusted 47 mm GF/Cs, quick-frozen on an aluminum block (pre-cooled to -80°C) and stored at -80°C. Later, filters were partially thawed and 48 *Calanus hyperboreus* (females, CV, CIV, CIII) were removed, placed in 2 ml chloroform and total lipids extracted (see below). Following Ohman (1997), lipids were dried, re-suspended in hexane, and applied to a combusted glass column (length=16.5 cm, internal diameter=1

cm, glass wool packed into tip) with a 6 ml bed volume of activated (110°C, 1 h) 100-200 mesh silica gel (~2.7 g). To clean the gel and remove hydrocarbons, the column was rinsed with ~30 ml 100% hexane. Total wax esters (WE: double peak) were then eluted with 45 ml 1% diethyl ether in hexane, dried under a stream of $N_2$, and weighed to constant mass. Following a modified version of Saito and Kotani (2000), the two components of the double WE peak isolated from *C. hyperboreus* were then separated. Using the same column described above, the silica gel was cleaned with 18 ml (3 bed volumes) 1:3 dichloromethane:hexane, eluted WE-I with 18 ml 1:2 dichloromethane:hexane and WE-II with 18 ml 1:1 dichloromethane:hexane. Lipids in all fractions were identified using thin-layer chromatography and portions reserved for fatty acid analysis (see below).

*3.3.4 Lipid analysis*

Lipids were extracted following a modified version of Folch et al. (1957). Samples were ground by hand using a metal rod, sonicated (4 min) and centrifuged (125 x g; 2 min) four times in a 8:4:3 chloroform:methanol:water mixture, and the organic layers removed and pooled. To separate and quantify lipid classes, samples were manually spotted on silica-coated Chromarods (SIII) and passed through the flame ionization detector (FID) of an Iatroscan MK V. The air and hydrogen flow rates were set to 2 l $min^{-1}$ and 190 ml $min^{-1}$, respectively. Rod development was done following Parrish (1987) where wax esters were resolved by double development in a non-polar solvent system (hexane:diethyl ether:formic acid (99:1:0.05), 25 min + 20 min). Although this

method does not allow for the separation of wax and steryl esters, preliminary analysis (short column gas chromatography) of total WE isolated from *Calanus hyperboreus* showed that the double peak resolved by the Iatroscan is composed of many wax ester species, but contains no steryl esters (Kehoe 2003).

The following standards (from Sigma-Aldrich Canada Ltd. unless otherwise stated) were used to calibrate the Iatroscan and establish peak identities: n-nonadecane (hydrocarbon, HC), total wax ester (double peak) isolated from *C. hyperboreus* (wax and steryl esters, WE/SE), 3-hexdecanone (ketone, KET), tripalmitin (triacylglcerol, TG), palmitic acid (free fatty acid, FFA), 1-hexadecanol (alcohol, ALC), cholesterol (sterol, ST), 1-monopalmitoyl-rac-glycerol (acetone-mobile polar lipid (AMPL); diacylglycerol (DG)), DL-α-phosphatidylcholine dipalmitoyl (phospholipid, PL). The relative amount (%) of each lipid class was determined as a function of total lipid (i.e., the sum of all lipid classes detected).

Fatty acids were quantified as methyl esters by FID using a Varian Model 3400 gas chromatograph (GC), following total lipid derivatization of samples with $BF_3$-methanol (85°C, 1 h). Methyl esters were analyzed on an Omegawax column following Budge and Parrish (1998). Tricosanoic acid was used as an internal standard, at a concentration of ~10% total fatty acids. Peaks were identified by comparing sample retention times to those of commercial standard mixtures (37-component, PUFA No. 1, PUFA No. 3, Bacterial Acid Methyl Esters Mix, Supelco (Sigma-Aldrich)) following

Ackman (1986), and by using a Varian 2000 GC/mass spectrometer. Lipid peaks that appeared abnormal due to contamination or machine error (e.g., spiking) were discarded and excluded from analyses. The term "odd and/or branched fatty acids" (OBFA) is used to describe those fatty acids that have odd-numbered carbon chains (with the exception of 21:5(n-3)) and/or iso and anteiso branches. This grouping therefore encompasses certain cyclic (e.g., ∇19:0), saturated (e.g., 15:0), branched (e.g., i-16:0) and monounsaturated (e.g., 17:1) fatty acids. The broader term "bacterial fatty acids" includes OBFA as well as other diagnostic bacterial fatty acids (i.e., 18:1(n-7)). Relative amounts (%) of all fatty acids were determined by comparing individual to total (sum of all detected acids) peak areas.

*3.3.5 Statistical analyses*

Changes in lipids during the acclimation period (day 0-23) were described using model-I regression analyses (S-Plus 2000). Histograms of residuals were identical in shape and distribution before and after arcsine transformation of response variables (percentage data). Therefore, all testing was done on untransformed numbers. Correlation analyses (Pearson, 2-tailed; SPSS 9.0) were used to determine the relationship between unsaturation coefficients and (1) polyunsaturated fatty acids and (2) the sum of saturates and monounsaturates in total lipid extracts. For all analyses, the rejection criterion was set to $\alpha$=0.05.

## 3.4 Results

### *3.4.1 The unsaturation coefficient: a wax ester-based omnivory index*

Total wax ester isolated from *Calanus hyperboreus* collected in the North Water Polynya was 95% pure (5% contamination with HC, AMPL and PL; data not shown) and appeared as a double peak on the Iatroscan (Figure 3.1a). Four other fractions collected from the column (18 ml 100% hexane (HC); 30 ml 5% diethyl ether (no lipid); 30 ml 100% diethyl ether (TG and polar lipids); 30 ml 100% methanol (PL)) and analyzed by Iatroscan revealed no WE, confirming that all recovered WE eluted in one fraction. Aside from minor HC contamination, the WE-I fraction contained only WE-I, while some WE-I (9% of total WE peak area) remained in the WE-II fraction (Figure 3.1b,c).

Total WE of *C. hyperboreus* was composed almost entirely of equal proportions of monounsaturated (MUFA; 47%) and polyunsaturated (PUFA; 47%) fatty acids (Table 3.2). A small percentage of saturated fatty acids (SFA; 6%) made up the rest of the total. Principal fatty acids were 16:1(n-7), 20:1(n-9), 20:5(n-3) and 22:1(n-1). The acyl component of WE-I was predominantly monounsaturated (88%) and saturated (10%). Major MUFA were 16:1(n-7), 20:1(n-9) and 22:1(n-11), while 14:0 and 16:0 were the dominant SFA. In WE-II, most of the fatty acids were polyunsaturated (83%; most of which was 20:5(n-3)) and monounsaturated (15%; mainly 16:1(n-7)). Odd and/or branched fatty acids (OBFA) constituted < 1% total fatty acids in all WE fractions.

The lipid compositions of WE-I and WE-II illustrate a distinct separation of PUFA and other fatty acids (SFA, MUFA) during thin-layer chromatography on Chromarods. The ratio between WE-II and total WE, hereafter referred to as the unsaturation coefficient (UC), is therefore proposed as an appropriate index of omnivory. Copepods feeding primarily on PUFA-rich phytoplankton are predicted to have a higher UC than those that with an omnivorous diet that includes bacterivorous ciliates and marine snow.

The estimates of UC agreed well with independent GC analyses when all *Calanus glacialis* (CV) lipid samples from the current study were considered. A reasonably strong, positive correlation ($r=0.66$, $p<0.01$) was found between UC and the percentage of PUFA in copepods fed during the incubation experiment (Figure 3.2a). Conversely, UC was negatively correlated ($r=-0.67$, $p<0.01$) with the sum of MUFA and SFA (Figure 3.2b) in *C. glacialis*. Even though I correlate total fatty acids (x-axes) with fatty acids in WE alone (using UC as a proxy; y-axes), 80 - ~100% of the fatty acids in these samples originated from this lipid class.

*3.4.2 Lipid composition of cultures*

The lipid class compositions of the two cultures were similar in terms of relative contributions of HC (~2%), SE/WE, KET, ALC (< 1%), and DG (4-8%) to total lipid (Table 3.3). *Thalassiosira hispida* contained an excess of FFA (37%), possibly as a result of lipolytic enzymes in the diatoms that release fatty acids from acyl lipid classes. A

boiling water treatment, which de-activates these enzymes (*cf.* Budge and Parrish 1999), significantly reduced FFA (by 78%) and AMPL (by 48%) levels in *T. hispida* (Parrish et al. unpublished data). In exponential phase cultures of this species, FFA and AMPL accumulate as TG is hydrolyzed (Parrish et al. unpublished data). The TG, FFA and AMPL levels were therefore corrected accordingly (Table 3.3). Corrected FFA and AMPL values for the diatom were similar to those of *Oxyrrhis marina* (FFA: 8 *vs.* 4%; AMPL: 15 *vs.* 22%). The major difference between the two cultures was that *O. marina* had a higher proportion of PL (38 *vs.* 6%) and lower proportions of TG (22 *vs.* 49%) and ST (2 *vs.* 14%).

Compared to the diatom, *Oxyrrhis marina* contained higher proportions of OBFA (13 *vs.* 3%) and SFA (48 *vs.* 17%), lower proportions of PUFA (31 *vs.* 59%) and similar proportions of MUFA (21 *vs.* 24%) (Table 3.4). In terms of individual fatty acids, each culture was characterized by distinct, diagnostic fatty acids. In *O. marina* these were i-15:0, ai-15:0, 16:0, 18:1(n-7), 18:2(n-6) and 22:6(n-3), and in *Thalassiosira hispida*, 16:1(n-7), 16:2(n-4), 16:3(n-4), 16:4(n-1), 18:4(n-3) and 20:5(n-3).

*3.4.3 Feeding physiology and changes in lipids during the incubation*

Clearance rates of *Calanus glacialis* were low ($\leq$ 2 ml copepod$^{-1}$ h$^{-1}$) but positive during most measurement intervals of the incubation (Figure 3.3a). After some of the animals were switched to *Thalassiosira hispida* (day 23), clearance rates were higher (relative to copepods fed *Oxyrrhis marina*), but this trend was not sustained. During the

second half of the incubation, clearance rates generally increased and became more variable. Copepods eating *O. marina* produced pink faecal pellets while those eating *T. hispida* produced brown pellets. Copepods usually consumed less than 70% of the cells provided at t=0 during each measurement interval (Figure 3.3b); the percentage eaten rose to 76-90% during measurement intervals (day 23-26, day 26-29) where relatively high clearance rates on *T. hispida* were coupled with moderate copepod densities. Copepod mortality generally increased during the incubation, with no obvious difference between control and treatment (Figure 3.3c).

Experimental copepods contained mostly WE (mean=87-96% of total lipid; Figure 3.4). Across all samples, AMPL, TG and PL were the next most important lipid classes, ranging between 0.5 and 4.8%, 0.3 and 5.4%, and 0.0 and 3.6% of total lipid, respectively. Proportions of HC, FFA, ALC and ST did not exceed 3% of total lipid in any sample. Except for minor fluctuations, the lipid class composition remained relatively unchanged during the acclimation period, although proportions of WE increased slightly and those of TG decreased slightly. The lipid class compositions of treatment and control samples were somewhat different. On days 35 and 42, treatment samples had relatively high proportions of ST, TG and FFA (compared to day 29), while in controls, these lipid classes were almost absent. During the 3-week acclimation period, copepod total lipid (TL: μg copepod$^{-1}$) decreased by about 30% and total fatty acids (TFA; μg copepod$^{-1}$) by about 45% (Table 3.5, Figure 3.5). Although regression

coefficients describing TL and TFA loss were significant, they accounted for little of the total variance (18-20%).

Principal fatty acids in the total lipid of *Calanus glacialis* during the incubation experiment (all samples) were 14:0, 16:0, 16:1(n-7), 20:1(n-9), 20:5(n-3) and 22:1 ((n-11)+(n-9)+(n-7); (n-11) main isomer) (Table 3.5). Absolute amounts of all fatty acids ($\mu$g copepod$^{-1}$) were determined, but because they were highly variable and overall trends much less obvious, only relative data are reported. Despite the high OBFA content in *Oxyrrhis marina*, neither the total ($\Sigma$OBFA; Figure 3.5), nor any of the eight individual fatty acids detected (Table 3.5), including potential biomarkers i-15:0 and ai-15:0 (Figure 3.6), increased during the acclimation period. Total SFA ($\Sigma$SFA) increased significantly ($r^2$=0.34, $p<0.001$), as well as the proportion of 16:0, although very weakly ($r^2$=0.12, $p=0.03$). Although the apparent increase in $\Sigma$MUFA during the acclimation period was not significant (Figure 3.5), proportions of 16:1(n-7) and 18:1(n-7) increased very weakly ($r^2$=0.11, $p=0.04$) and moderately ($r^2$=0.44, $p<0.0001$), respectively (Figure 3.6).

$\Sigma$PUFA decreased significantly during the acclimation period ($r^2$=0.30, $p<0.001$; Figure 3.5) probably as a result of the steady decline in the proportion of the dominant fatty acid 20:5(n-3) ($r^2$=0.40, $p<0.0001$; Figure 3.6). Additionally, proportions of 16:3(n-4), 16:4(n-1) and 18:4(n-3) decreased significantly ($p\leq0.0001$) during this 3-week period ($r^2$=0.35, 0.53, 0.62, respectively). Proportions of 16:2(n-4), 18:2(n-6) and

22:6(n-3) were unrelated to day. Unsaturation coefficients declined steadily during the acclimation period ($r^2$=0.43, $p<0.0001$) (Figure 3.5).

Due to unexpectedly high rates of mortality, copepods were soon depleted, shortening the treatment period considerably. Lack of sufficient data and high variability during the 3-week treatment period (day 29-42) may have obscured any significant relationships between lipids and day caused by the diet switch. However, several interesting trends emerged in the data. In *C. glacialis* treatment samples, UC, ΣPUFA and proportions of 16:1(n-7), 16:2(n-4), 16:3(n-4), 16:4(n-1) and 20:5(n-3) were higher and ΣMUFA lower relative to control samples at day 42 (Table 3.5, Figure 3.5, Figure 3.6). After the switch, TL and TFA remained relatively constant.

## 3.5 Discussion

### *3.5.1 The effect of copepod physiological state on lipid turnover*

The copepods used in the incubation experiment were relatively healthy, were provided with suitable food at adequate concentrations, and fed on both prey actively and without obvious preference. However, the clearance rates reported here were at the low end of those estimated for pre-, mid-, and post-bloom *Calanus glacialis* CV from Arctic waters (estimated by gut fluorescence (Tande and Båmstedt 1985; Hansen et al. 1990) or in "simulated" *in situ* incubations (Conover et al. 1991)). In Conception Bay, Newfoundland, diapause in *Calanus* spp. begins around August (Davis 1982; Deibel et al. unpublished data), which coincides with the collection time and explains the

dominance of late copepodite stages (data not shown) deep in the water column (animals at or below 225 m).

According to Hirche (1996), diapause in *Calanus finmarchicus* (usually as copepodite stage V) is characterized by five phases: preparatory (accumulation of lipid, seasonal descent), induction (reduced digestive tract and metabolism), refractory (torpidity, incapacity to develop), activation (gonadogenesis, competency to develop), and termination (moulting, seasonal ascent). The normal swimming behavior, occasional moulting to adulthood (data not shown), and the active but slow feeding activity observed in the incubations suggests that the experimental animals were in the early stages of diapause, probably the induction phase. Cyclopoid copepods removed from active diapause *in situ* will eat during laboratory experiments, although feeding rates and digestive enzyme activities are much reduced (Krylov et al. 1996).

The lipid composition of wild *Calanus glacialis* (CV) from Conception Bay resembled those of winter-collected *Calanus* spp. from other marine systems (Lee 1974), which are relatively low in PUFA and high in 20:1(n-9) and 22:1(n-11). During times of low food availability, these highly energetic MUFA are preferentially conserved (Lee 1974) and elevated proportions have been used as indicators of food scarcity *in situ* (Kattner et al. 1989). It is uncertain whether these copepods were starving *in situ*, undergoing normal compositional changes during early diapause, or were kept too long without food before the experiment began (16 d). Despite the lengthy starvation period in

the laboratory, animals appeared healthy when the experiment began (e.g., active swimming behavior, low mortality, conspicuous oil sacs). Wild-caught *C. glacialis* had probably fed recently on diatoms given that proportions of diagnostic biomarkers (16:1(n-7), 16:4(n-1), 20:5(n-3)) were relatively high whereas those of dinoflagellates (18:4(n-3), 22:6(n-3); Viso and Marty 1993) were low.

The fatty acid composition of the heterotrophic dinoflagellate *Oxyrrhis marina* varies depending on its diet. *O. marina* fed on cryptophytes and chlorophytes contain large proportions of 16:0 and 22:6(n-3) and smaller proportions of 14:0, 18-carbon PUFA and 20:5(n-3) (Klein Breteler et al. 1999). The bacterivorous strain (CCAP 1133/4) used in this study also contained high proportions of 16:0 and 22:6(n-3), in addition to i-15:0, ai-15:0, and 18:1(n-7) transferred directly from its bacterial prey. Preliminary lipid analysis of the bacteria fed to *O. marina* showed that they are composed primarily of i-15:0, ai-15:0, 16:0 and 18:1(n-7) (73% total fatty acids: Stevens et al. unpublished data). Interestingly, *O. marina* is capable of synthesizing large amounts of essential fatty acids (22:6(n-3) and to a lesser extent 20:5(n-3)), despite their complete absence in the diet (none in prey used by us or by Klein Breteler et al. 1999).

*Calanus glacialis* fed *Oxyrrhis marina* for 3 weeks developed an omnivorous lipid composition relative to the wild-captured copepods. Notably, UC decreased as copepods ate the relatively PUFA-poor prey and proportions of the bacterial fatty acid 18:1(n-7) increased. The decrease in UC was probably due mainly to loss of PUFA,

specifically 20:5(n-3), and less to increases in proportions of dominant MUFA and SFA (i.e., 16:1(n-7), 16:0) that were only weakly associated with day during the acclimation period. In addition, absolute amounts (μg copepod$^{-1}$) of many PUFA declined whereas SFA and MUFA remained constant (data not shown). The overall magnitude of the fatty acid compositional changes seen here was much reduced compared to Graeve et al. (1994), who found marked responses in biomarker lipids in *Calanus* spp. as a result of diet (increases/decreases of up to 20%). It is also unusual that proportions of i-15:0, ai-15:0 and 22:6(n-3), all elevated in *O. marina*, did not change in *C. glacialis*. The lowered metabolic state of the copepods may have affected the incorporation rates of all fatty acids, some more than others.

Severe starvation in copepods is characterized by major losses of storage relative to structural lipids (Lee et al. 1970), which could result in elevated levels of membrane lipids (such as 20:5(n-3) and 22:6(n-3) which are major PL constituents in *Calanus* spp. (Lee et al. 1971)). Neither indication of severe starvation was observed here: lipid class compositions and total proportions of storage lipid (WE + TG) in *C. glacialis* changed little during the incubation and proportions of 20:5(n-3) and ΣPUFA declined. Although proportions of TG declined steadily during the acclimation period, this lipid class is catabolized very quickly in copepods, compared to WE, and may not be a good indicator of major starvation (Sargent et al. 1976). While *C. glacialis* lost significant absolute amounts of lipid, TL loss in this experiment (~32%) was comparable to or less than that observed by Graeve et al. (1994) for healthy (very low mortality, M. Graeve, personal

communication), actively feeding *C. finmarchicus* CV (40% loss of TL) and *C. hyperboreus* CV (60% loss).

Although treatment effects were not significant, the observed trends (apparent increases in UC and proportions of ΣPUFA, 16:1(n-7), 16:2(n-4), 16:3(n-4), 16:4(n-1) and 20:5(n-3) in treatment samples) support the view that the lipid composition of *Calanus glacialis* changed in response to diet. The increase in proportions of TG and ST, rich in *T. hispida*, in treatment samples was further evidence that copepods were incorporating dietary lipid despite their physiological state. Therefore, the changes in *C. glacialis* lipids during the experiment, particularly the decrease in UC in animals fed *Oxyrrhis marina*, were most likely the result of diet and not starvation.

### *3.5.2 Utility of unsaturation coefficients and bacterial fatty acids to* Calanus *spp.*

The splitting of WE into two peaks during thin-layer chromatography will occur (using a non-polar solvent system (see Methods; Oshima et al. 1987)) if there is a bimodal distribution in WE acyl components in terms of unsaturation (Parrish et al. 1992). Wax ester splitting was shown here for Arctic *Calanus hyperboreus*, *C. glacialis* and *Metridia longa* (Figure 3.1) and in *C. glacialis* collected in Conception Bay, Newfoundland. All three species store large amounts of PUFA-rich WE (Sargent and Henderson 1986). The fatty acid analysis of each WE peak, confirmed that the first peak, WE-I, is composed almost entirely of MUFA + SFA (98%), while WE-II contains mostly PUFA (83%) with a small amount of MUFA. Similar results were found by Saito and

Kotani (2000): WE-I in three calanoid copepods (*Neocalanus cristatus*, *N. flemingeri*, *M. okhotensis*) caught in the northwestern Pacific was composed of between 79 to 93% MUFA + SFA, while the proportion of PUFA in WE-II ranged from 55 to 88%. In agreement with Oshima et al. (1987), the WE splitting observed was the result of varying degrees of unsaturation of acyl groups and not chain length.

Typically, lipid classes are separated and quantified using an Iatroscan (silica-gel chromatography on Chromarods with flame-ionization detection) or silica-coated plates (quantification by densitometer). The former system is superior in that its partial scanning facility permits extensive analysis of each sample (e.g., quantitation of 10+ lipid classes at once), thereby identifying all peaks with greater confidence (reviewed in Parrish et al. 2000). Unsaturation coefficients can thus be quickly obtained using an Iatroscan, providing a robust and easily replicable estimate of copepod feeding history *in situ*.

An obvious shortfall of the UC is that it is affected by catabolic processes, such as lipid mobilization during diapause for nutrition and gonadogenesis, where PUFA may be preferentially metabolized (see references above). UC may thus be most useful during the active growing season of copepods: after spawning and before diapause. Another consideration is the degree of unsaturation of microzooplankton fatty acids (for which there is very little data). Although bacterivorous ciliates contain less PUFA than phytoplankton, herbivorous species can contain significant amounts, more so than diatoms and dinoflagellates (Claustre et al. 1989: %PUFA: tintinids=59,

dinoflagellates=42, diatoms=9). It is possible that UC will be most appropriate as an omnivory index in instances where available prey are divergent in their PUFA content.

These data suggest that OBFA are not useful in tracking the transfer of microbial prey to *Calanus* spp. The small levels of these fatty acids detected in *C. glacialis* could be the result of bacteria adhering to external surfaces (Huq et al. 1983) or they might represent enteric bacteria (Nagasawa and Nemato 1988). However, OBFA in Arctic *C. hyperboreus* (CV), *C. glacialis* (CV) and *Metridia longa* (CVI females) appeared in neutral but not polar lipid (Stevens et al. unpublished data), suggesting that the signals had been assimilated and did not represent intact cells (bacterial lipids are predominantly polar; Gérin and Goutx 1993). Since OBFA levels in *Oxyrrhis marina* were relatively high and no transfer was detected, it could mean that the quota for these molecules had already been reached. It is unlikely that these fatty acids are quickly modified by copepods given that they are more resistant to breakdown in sediments than are PUFA (Haddad et al. 1991). The lack of transfer may once again relate to the physiological state of the copepods, in which case such an experiment should be repeated with animals collected during mid-bloom conditions when digestive enzymes and feeding potential are at a maximum (Hassett and Landry 1990).

On the other hand, the bacterial fatty acid 18:1(n-7), which is abundant in *Oxyrrhis marina* (15% of total), was steadily incorporated by copepods eating these cells. Pranal et al. (1996) suggested that high levels of 18:1(n-7) (up to 18%) in gastropods

from a hydrothermal vent community were the result of assimilation of lipid produced by endogenous sulfur-oxidizing bacteria. Similar conclusions were drawn to explain high proportions of 18:1(n-7) in hydrothermal vent and littoral bivalves (Ben-Mlih et al. 1992; Zhukova et al. 1992). This bacterial fatty acid may prove useful in demonstrating ingestion of microbially-based prey by *Calanus* spp. *in situ*.

## 3.6 Conclusions

A newly developed wax ester-based omnivory index (unsaturation coefficient) can quickly deduce the diet histories and feeding strategies of wax ester-storing copepods. The bacterial fatty acid 18:1(n-7) may be useful in demonstrating connections between *Calanus* spp. and the microbial food web. Further understanding of the *in vivo* turnover of dietary fatty acids could lead to a quantitative method for estimating true *in situ* omnivorous ingestion rates of zooplankton (*versus* those derived during "simulated" *in situ* incubations).

## 3.7 Acknowledgements

The author would like to thank the officers and crew of the *N.G.C.C. Pierre Radisson* and the *Karl and Jackie II* for their assistance in the field. Sing-Hoi Lee, Betty Hatfield, Lilia Jackman, Louise Copeman, Cynthia McKenzie and Ray Thompson helped greatly with methodology and equipment. This research was supported by an NSERC grant awarded to D. Deibel, R.B. Rivkin and M.R. Anderson (NOW polynya project) and the Discovery Grant of D. Deibel.

## 3.8 References


Ackman RG (1986) WCOT (Capillary) Gas-liquid chromatography. In: Hamilton RJ, Rossell JB (eds) Analysis of Oils and Fats. Elsevier, London, pp 137-206

Ben-Mlih F, Marty J-C, Fiala-Médioni A (1992) Fatty acid composition in deep hydrothermal vent symbiotic bivalves. J Lipid Res 33:1797-1806

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29: 1547-1559

Budge SM, Parrish CC (1999) Lipid class and fatty acid composition of *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia pungens* and effects of lipolytic enzyme deactivation. Phytochem 52:561-566

Claustre H, Marty J-C, Cassiani L, Dagaut J (1989) Fatty acid dynamics in phytoplankton and microzooplankton communities during a spring bloom in the coastal Ligurian Sea: ecological implications. Mar Microb Food Webs 3:51-66

Conover RJ, Harris LR, Bedo AW (1991) Copepods in cold oligotrophic waters - how do they cope? Bull Plankton Soc Japan (Spec Vol): 177-199

Cripps GC, Hill HJ (1998) Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. Deep-Sea Res I 45: 1357-1381

Davis CC (1982) A preliminary quantitative study of the zooplankton from Conception Bay, Insular Newfoundland, Canada. Int Rev Gesamten Hydrobiol 67:713-747

Desvilettes Ch, Bourdier G, Breton JC, Combrouze Ph (1994) Fatty acids as organic markers for the study of trophic relationships in littoral cladoceran communities. J Plankton Res 16: 643-659

Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limnol Oceanogr 40:860-867

Falk-Petersen S, Sargent JR, Lønne OJ, Timofeev S (1999) Functional biodiversity of lipids in Antarctic zooplankton: *Calanoides acutus*, *Calanus propinquus*, *Thysanoessa macrura* and *Euphausia crystallorophias*. Polar Biol 21:37-47

Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17: 805-815

Gérin C, Goutx M (1993) Separation and quantification of phospholipids from marine bacteria with the Iatroscan Mark IV TLC-FID. J Planar Chromatogr 6: 307-312

Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97-110

Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229-239

Haddad RI, Martens CS, Farrington JW (1991) Quantifying early diagenesis of fatty acids in a rapidly accumulating coastal marine sediment. Adv Org Geochem 19:205-216

Hansen B, Berggreen UC, Tande KS, Eilertsen HC (1990) Post-bloom grazing by *Calanus glacialis*, *C. finmarchicus* and *C. hyperboreus* in the region of the Polar Front, Barents Sea. Mar Biol 104: 5-14

Harvey HR, Ederington MC, McManus GB (1997) Lipid composition of the marine ciliates *Pleuronema* sp. and *Fabrea salina*: shifts in response to changes in diet. J Eukaryot Microbiol 44: 189-193

Hassett RP, Landry MR (1990) Seasonal changes in feeding rate, digestive enzyme activity, and assimilation efficiency of *Calanus pacificus*. Mar Ecol Prog Ser 62:203-210

Hirche H-J (1996) Diapause in the marine copepod, *Calanus finmarchicus* - a review. Ophelia 44: 129-143

Huq A, Small EB, West PA, Huq I, Rahman R, Colwell RR (1983) Ecological relationships between *Vibrio cholerae* and planktonic crustacean copepods. Appl Environ Microbiol 45:275-283

Kattner G, Hirche HJ, Krause M (1989) Spatial variability in lipid composition of calanoid copepods from Fram Strait, the Arctic. Mar Biol 102:473-480

Kehoe JJ (2003) Total marine lipid profiling by short column gas chromatography. MS thesis, Memorial University of Newfoundland, St. John's

Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. Mar Biol 135: 191-298

Krylov PI, Alekseev VR, Frenkel OA (1996) Feeding and digestive activity of cyclopoid copepods in active diapause. Hydrobiologia 320:71-79

Lee RF, Nevenzel JC, Paffenhöfer G-A, Benson AA (1970) The metabolism of wax esters and other lipids by the marine copepod, *Calanus helgolandicus*. J Lipid Res 11:237-240

Lee RF, Nevenzel JC, Paffenhöfer G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9:99-108

Lee RF (1974) Lipid composition of the copepod *Calanus hyperboreus* from the Arctic ocean. Changes with depth and season. Mar Biol 26:313-318

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204:65-77

Marin V, Huntley ME, Frost BW (1986) Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. Mar Biol 93:49-58

Meziane T, Bodineau L, Retiere C, Thoumelin G (1997) The use of lipid markers to define sources of organic matter in sediment and food web of the intertidal salt-marsh-flat ecosystem of Mont-Saint-Michel Bay, France. J Sea Res 38:47-58

Meziane T, Tsuchiya M (2000) Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar Ecol Prog Ser 200:49-57

Nagasawa S, Nemato T (1988) Presence of bacteria in guts of marine crustaceans and on their fecal pellets. J Plankton Res 10:559-564

Ohman MD (1997) On the determination of zooplankton lipid content and the occurrence of gelatinous copepods. J Plankton Res 19:1235-1250

Ohshima T, Ratnayake WMN, Ackman RG (1987) Cod lipids, solvent systems and the effect of fatty acid chain length and unsaturation on lipid class analysis by Iatroscan TLC-FID. Amer Oil Chem Soc 64:219-223

Parrish CC (1987) Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can J Fish Aquat Sci 44:722-731

Parrish CC, Bodennec G, Gentien P (1992) Separation of polyunsaturated and saturated lipids from marine phytoplankton on silica gel-coated Chromarods. J Chromatogr 607:97-104

Parrish CC, Abrajano TA, Budge SM, Helleur RJ, Hudson ED, Pulchan K, Ramos C (2000) Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky P (ed) The Handbook of Environmental Chemistry (Vol. 5). Springer-Verlag, Berlin, pp 193-223

Pranal V, Fiala-Médioni A, Guezennec J (1996) Fatty acid characteristics in two symbiotic gastropods from a deep hydrothermal vent of the West Pacific. Mar Ecol Prog Ser 142:175-184

Runge JA, de LaFontaine Y (1996) Characterization of the pelagic ecosystem in surface waters of the northern Gulf of St. Lawrence in early summer: the larval redfish-*Calanus*-microplankton interaction. Fish Oceanogr 5:21-37

Saito H, Kotani Y (2000) Lipids of four boreal species of calanoid copepods: origin of monoene fats of marine animals at higher trophic levels in the grazing food chain in the subarctic ocean ecosystem. Mar Chem 71:69-82

Sargent JR, Lee RF, Nevenzel JC (1976). Marine Waxes. In: Kolattukudy PE (ed) Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, pp 49-91

Sargent JR, Eilersten HC, Falk-Petersen S, Taasen JP (1985) Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. Mar Biol 85:109-116

Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) The Biological Chemistry of Marine Copepods. Clarendon Press, Oxford, pp 59-108

Tande KS, Båmstedt U (1985) Grazing rates of the copepods *Calanus glacialis* and *C. finmarchicus* in arctic waters of the Barents Sea. Mar Biol 87:251-258

Viso A-C, Marty J-C (1993) Fatty acids from 28 marine microalgae. Phytochem 34:1521-1533

Zhukova NV, Kharlamenko VI, Svetashev VI, Rodionov IA (1992) Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. J Exp Mar Biol Ecol 162:253-263

## 3.9 Tables

Table 3.1. Type, chemical composition and concentration of prey fed to *Calanus glacialis* (CV) during a 6-week incubation (error estimates represent one standard deviation).

| Culture | Type | Source | Size (μm) | Mean lipid content[a] (pg cell$^{-1}$) | Mean carbon content[b] (pg cell$^{-1}$) | Mean cells provided[a,c] (cells ml$^{-1}$ day$^{-1}$) | Mean carbon provided[a,c] (μg C l$^{-1}$ day$^{-1}$) |
|---|---|---|---|---|---|---|---|
| *Thalassiosira hispida* | chain-forming centric diatom | local isolate (coastal NL) | 15-25 (diameter) | 123.2 ± 70.1 (n=5) | 174.3 ± 3.3 (n=3) | 2963 ± 588 (n=18) | 516 ± 103 (n=18) |
| *Oxyrrhis marina* (Strain 1133/4) | bacterivorous dinoflagellate | CCAP (Oban) | 21-25 (l) 13-15 (w) | 100.6 ± 44.8 (n=14) | 424.2 ± 33.3 (n=4) | 918 ± 259 (n=60) | 390 ± 110 (n=60) |

[a]include all measurements taken throughout experiment, reflecting changes in culture growth

[b]*T. hispida*: samples taken in triplicate from 1 culture flask; *O. marina*: samples taken in duplicate from 2 separate culture flasks

[c]based on prey provided once, at the beginning of each 3-4 day measurement interval

Table 3.2. Relative fatty acid composition (%) of total wax ester (WE), WE-I and WE-II isolated from *Calanus hyperboreus* collected in September in the North Water Polynya.

| Fatty Acid | Total WE | WE-I | WE-II |
|---|---|---|---|
| 14:0 | 3.03 | 5.96 | 0.66 |
| 14:1 | 0.22 | 0.30 | 0.16 |
| i-15:0 | 0.10 | 0.16 | 0.05 |
| ai-15:0 | 0.04 | 0.08 | nd |
| 15:0 | 0.05 | 0.11 | nd |
| 15:1 | 0.10 | 0.15 | 0.08 |
| 16:0 | 1.51 | 3.60 | 0.31 |
| 16:1(n-7) | 22.94 | 37.97 | 10.86 |
| 16:2(n-4) | 1.62 | 1.25 | 1.92 |
| 16:3(n-4) | 1.58 | 0.21 | 3.00 |
| 16:4(n-3) | 0.22 | nd | 0.42 |
| 16:4(n-1) | 5.14 | nd | 9.84 |
| ai-17:0 | 0.42 | 0.23 | 0.48 |
| 17:1 | 0.09 | nd | 0.08 |
| 18:0 | 0.14 | nd | nd |
| 18:1(n-9) | 2.04 | 4.35 | 0.81 |
| 18:1(n-7) | 1.70 | 3.77 | 0.17 |
| 18:1(n-5) | 0.36 | nd | 0.05 |
| 18:2(n-6) | c | nd | c |
| 18:2(n-4) | c | nd | 0.46 |
| 18:3(n-6) | 0.99 | nd | 0.83 |
| 18:3(n-4) | 0.69 | nd | 0.21 |
| 18:3(n-3) | 0.70 | nd | 0.40 |
| 18:4(n-3) | 2.45 | nd | 3.41 |
| 18:4(n-1) | 1.04 | nd | 1.55 |
| 20:0 | 0.35 | nd | 0.18 |
| 20:1(n-11) | 0.14 | 1.30 | nd |
| 20:1(n-9) | 8.55 | 17.71 | 1.50 |
| 20:1(n-7) | 1.84 | 4.00 | 0.44 |
| 20:4(n-6) | c | nd | c |
| 20:4(n-3) | 0.46 | nd | 0.85 |
| 20:5(n-3) | 27.57 | nd | 52.14 |
| 21:5(n-3) | 0.12 | nd | 0.22 |
| 22:1(n-11) | 8.01 | 16.32 | 0.85 |
| 22:1(n-9) | 0.90 | 1.93 | 0.29 |
| 22:1(n-7) | 0.17 | 0.31 | nd |
| 22:5(n-3) | 1.79 | nd | 3.28 |
| 22:6(n-3) | 2.63 | nd | 4.49 |
| 24:1 | 0.13 | 0.30 | nd |
| | | | |
| ΣOBFA | 0.79 | 0.72 | 0.69 |
| ΣSFA | 5.64 | 10.14 | 1.68 |
| ΣMUFA | 47.20 | 88.41 | 15.29 |
| ΣPUFA | 46.99 | 1.46 | 83.03 |

c=exclusion of data due to contamination (contaminated values ranged from 1.2-4.0% total fatty acids; these fatty acids rarely reach 1% of total fatty acids in total lipid fractions of *C. hyperboreus* collected over a broad geographical area in the North Water (see Appendices 7-8); nd=not detected

Table 3.3. Relative lipid class composition (% total lipid) of late exponential phase bacterivorous dinoflagellates and diatoms (numbers expressed as means ± 1 standard deviation).

| Lipid Class | *Oxyrrhis marina* (CCAP 1133/4) (n=14) | *Thalassiosira hispida* (local isolate) (n=5) |
|---|---|---|
| Hydrocarbons | 2.1 ± 1.6 | 1.8 ± 2.6 |
| Steryl/Wax esters | 0.6 ± 2.0 | 0.5 ± 1.2 |
| Ketones | 0.8 ± 1.1 | 0.6 ± 1.3 |
| Triacylglycerols | 22.3 ± 11.4 | 6.1 ± 4.9 (48.8%)[a] |
| Free fatty acids | 4.3 ± 4.6 | 36.6 ± 12.3 (8.1%)[a] |
| Alcohols | 0.1 ± 0.3 | 0.8 ± 1.1 |
| Sterols | 1.8 ± 2.4 | 14.4 ± 7.3 |
| Diacylglycerols | 7.7 ± 7.4 | 3.7 ± 2.3 |
| Acetone-mobile polar lipids | 22.2 ± 8.9 | 29.4 ± 3.4 (15.3%)[a] |
| Phospholipids | 37.5 ± 11.5 | 5.7 ± 2.2 |

[a]Corrected values (Parrish et al., unpublished) in brackets

Table 3.4. Relative fatty acid composition (%) of *Oxyrrhis marina* and *Thalassiosira hispida* cultures fed to *Calanus glacialis* (CV).

| Fatty Acid[a] | *Oxyrrhis marina* (n=14) | *Thalassiosira hispida* (n=6) |
|---|---|---|
| 14:0 | 6.51 ± 1.76 | 6.95 ± 1.46 |
| i-15:0 | 3.31 ± 1.57 | 0.30 ± 0.08 |
| ai-15:0 | 4.46 ± 0.56 | 0.08 ± 0.04 |
| i-16:0 | 0.85 ± 0.18 | 0.51 ± 0.17 |
| 16:0 | 29.01 ± 3.18 | 6.55 ± 0.38 |
| 16:1(n-7) | 2.26 ± 1.84 | 21.33 ± 7.85 |
| 16:2(n-4) | nd | 2.66 ± 0.47 |
| 16:3(n-4) | nd | 3.41 ± 0.85 |
| 16:4(n-1) | nd | 7.85 ± 3.58 |
| i-17:0 | 1.33 ± 0.66 | 0.74 ± 0.43 |
| ai-17:0 | 0.42 ± 0.10 | 0.70 ± 0.08 |
| 17:1 | 1.02 ± 0.14 | 0.01 ± 0.02 |
| 18:1(n-9) | 1.99 ± 0.36 | 1.15 ± 0.28 |
| 18:1(n-7) | 15.13 ± 2.23 | 0.10 ± 0.01 |
| 18:2(n-6) | 2.89 ± 0.61 | 0.37 ± 0.07 |
| 18:4(n-3) | nd | 5.45 ± 1.01 |
| ∇19:0 | 0.88 ± 0.14 | nd |
| 20:5(n-3) | 1.77 ± 0.69 | 34.58 ± 1.69 |
| 22:5(n-3) | 0.94 ± 0.21 | 0.01 ± 0.01 |
| 22:6(n-3) | 25.07 ± 5.17 | 2.15 ± 0.58 |
| | | |
| ΣOBFA | 12.82 ± 2.86 | 3.12 ± 0.91 |
| ΣSFA | 48.24 ± 5.85 | 16.53 ± 2.47 |
| ΣMUFA | 21.07 ± 4.19 | 24.14 ± 7.48 |
| ΣPUFA | 30.70 ± 5.45 | 59.34 ± 5.52 |

[a]data not shown where, in both cultures, OBFA were ~0.5 % of the total and even-numbered saturated, monounsaturated and polyunsaturated fatty acids were < 1 %; numbers expressed as means ± 1 standard deviation; nd=not detected

Table 3.5. Relative fatty acid composition (%), total lipid (TL), total fatty acids (TFA) and unsaturation coefficients (UC) in *Calanus glacialis* (CV) at various points during the 42-day incubation experiment.

| Lipid (%) | Wild (Day 0) | End of Acclimation (Day 23) | End of Incubation (Day 42) | |
|---|---|---|---|---|
| | | | Treatment | Control |
| 14:0 | 9.96 ± 1.77 | 11.08 ± 1.56 | 11.17 ± 0.78 | 10.59 ± 0.31 |
| 14:1 | 0.10 ± 0.03 | 0.20 ± 0.10 | 0.22 ± 0.07 | 0.16 ± 0.04 |
| i-15:0 | 0.13 ± 0.03 | 0.16 ± 0.06 | 0.21 ± 0.05 | 0.14 ± 0.04 |
| ai-15:0 | 0.07 ± 0.02 | 0.10 ± 0.03 | 0.13 ± 0.02 | 0.10 ± 0.03 |
| 15:0 | 0.25 ± 0.01 | 0.30 ± 0.07 | 0.30 ± 0.04 | 0.25 ± 0.01 |
| 15:1 | 0.12 ± 0.01 | 0.16 ± 0.05 | 0.14 ± 0.02 | 0.14 ± 0.00 |
| i-16:0 | 0.07 ± 0.06 | 0.06 ± 0.01 | 0.05 ± 0.00 | [a]0.06 |
| ai-16:0 | 0.03 ± 0.02 | 0.03 ± 0.00 | 0.02 ± 0.00 | [a]0.04 |
| 16:0 | 6.24 ± 0.25 | 7.27 ± 0.64 | 7.45 ± 1.73 | 5.60 ± 0.25 |
| 16:1(n-7) | 19.19 ± 1.85 | 21.92 ± 1.64 | 23.87 ± 0.56 | 21.64 ± 0.23 |
| 16:1(n-5) | 0.57 ± 0.06 | 0.62 ± 0.14 | 0.71 ± 0.06 | 0.63 ± 0.09 |
| 16:2(n-4) | 1.45 ± 0.11 | 1.46 ± 0.15 | 1.83 ± 0.17 | 1.68 ± 0.15 |
| 16:3(n-4) | 1.25 ± 0.25 | 0.77 ± 0.35 | 0.55 ± 0.15 | 0.25 ± 0.10 |
| 16:4(n-3) | 0.09 ± 0.03 | 0.09 ± 0.02 | 0.08 ± 0.01 | 0.05 ± 0.01 |
| 16:4(n-1) | 2.38 ± 0.62 | 0.72 ± 0.59 | 0.64 ± 0.24 | 0.14 ± 0.08 |
| i-17:0 | 0.17 ± 0.02 | 0.17 ± 0.03 | 0.21 ± 0.07 | 0.18 ± 0.04 |
| ai-17:0 | 0.35 ± 0.06 | 0.27 ± 0.08 | 0.37 ± 0.08 | 0.27 ± 0.02 |
| 18:0 | 0.33 ± 0.06 | 0.42 ± 0.11 | 0.32 ± 0.13 | 0.28 ± 0.04 |
| 18:1(n-9) | 2.66 ± 0.31 | 2.84 ± 0.34 | 2.78 ± 0.15 | 2.78 ± 0.08 |
| 18:1(n-7) | 0.92 ± 0.06 | 1.17 ± 0.16 | 1.31 ± 0.25 | 1.18 ± 0.16 |
| 18:1(n-5) | 0.35 ± 0.05 | 0.62 ± 0.20 | 0.62 ± 0.19 | 0.47 ± 0.04 |
| 18:2(n-6) | 0.65 ± 0.12 | 0.67 ± 0.21 | 0.68 ± 0.07 | 0.50 ± 0.01 |
| 18:2(n-4) | 0.15 ± 0.14 | 0.09 ± 0.17 | c | c |
| 18:3(n-6) | 0.21 ± 0.24 | 0.16 ± 0.14 | 0.06 ± 0.11 | c |
| 18:3(n-4) | 0.07 ± 0.12 | 0.04 ± 0.05 | 0.11 ± 0.03 | [a]0.06 |
| 18:3(n-3) | 0.17 ± 0.07 | 0.20 ± 0.04 | [a]0.17 | [a]0.10 |
| 18:4(n-3) | 2.46 ± 0.32 | 1.12 ± 0.46 | [a]0.79 | [a]0.74 |
| 18:4(n-1) | 0.39 ± 0.04 | 0.32 ± 0.05 | [a]0.30 | [a]0.34 |
| 20:0 | 0.08 ± 0.05 | 0.02 ± 0.03 | 0.02 ± 0.04 | 0.03 ± 0.05 |
| 20:1(n-9) | 20.51 ± 2.10 | 18.93 ± 2.76 | 18.90 ± 2.52 | 26.11 ± 2.98 |
| 20:5(n-3) | 11.06 ± 1.23 | 7.77 ± 1.75 | 10.29 ± 4.05 | 4.95 ± 2.08 |
| 21:5(n-3) | 0.29 ± 0.25 | 0.18 ± 0.16 | 0.21 ± 0.02 | 0.14 ± 0.07 |
| 22:1[c] | 14.68 ± 1.57 | 14.17 ± 3.48 | 12.36 ± 4.09 | 16.64 ± 1.86 |
| 22:5(n-3) | 0.65 ± 0.09 | 0.46 ± 0.03 | 0.72 ± 0.24 | [a]0.26 |
| 22:6(n-3) | 1.79 ± 0.28 | 3.29 ± 2.87 | 2.93 ± 1.90 | 2.90 ± 2.15 |
| 24:1 | 0.59 ± 0.18 | 1.23 ± 1.11 | 0.83 ± 0.25 | 0.62 ± 0.00 |
| ΣOBFA | 1.19 ± 0.12 | 1.23 ± 0.41 | 1.42 ± 0.24 | 1.15 ± 0.15 |
| ΣSFA | 16.91 ± 0.92 | 20.12 ± 2.05 | 20.25 ± 2.17 | 17.50 ± 0.13 |
| ΣMUFA | 59.69 ± 2.74 | 62.00 ± 4.43 | 61.47 ± 5.95 | 70.35 ± 1.32 |
| ΣPUFA | 22.64 ± 1.67 | 17.88 ± 4.28 | 18.28 ± 3.99 | 12.14 ± 1.45 |
| TL[b] | 619.29 ± 63.25 | 418.85 ± 77.27 | 432.0 ± 122.0 | 396.2 ± 148.7 |
| TFA[b] | 274.04 ± 37.83 | 150.65 ± 59.22 | 190.8 ± 78.5 | 228.2 ± 6.0 |
| UC | 0.18 ± 0.02 | 0.10 ± 0.02 | 0.09 ± 0.04 | 0.06 ± 0.03 |

[a]single values
[b]TL=total lipid (sum of all lipid classes); TFA=total fatty acids (sum of all fatty acids); units for both=μg copepod$^{-1}$
[c]the fatty acids 22:1(n-11) (major isomer), (n-9), and (n-7) were not always well separated so all were pooled
c=exclusion of data due to contamination (all excluded fatty acids (including 20:3(n-6), 20:3(n-3), 20:4(n-6), 20:4(n-3); indistinguishable from background noise) were found in small amounts in wild *Calanus glacialis* samples from the North Water Polynya (<1% TFA; see Appendices 9-10))

## 3.10 Figures

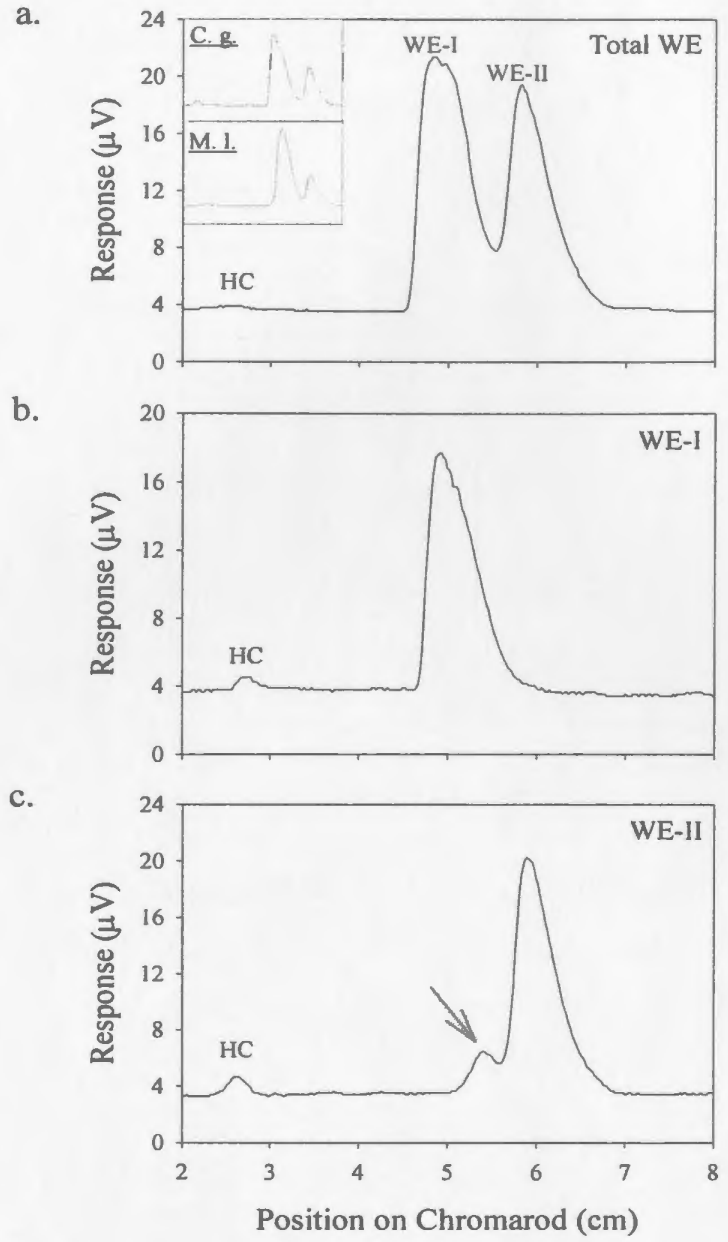
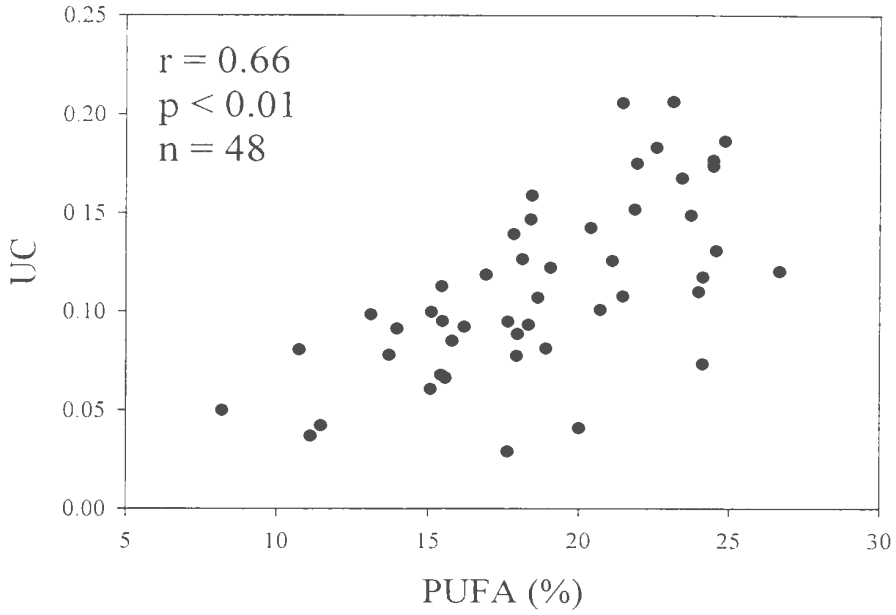



Figure 3.1. Iatroscan chromatograms of (a) the double wax ester (total WE) peak of WE-storing copepods from the North Water Polynya (main panel: *Calanus hyperboreus*; insets: *C. glacialis* and *Metridia longa*) and (b), (c) two chemically distinct WE components (I and II) in *C. hyperboreus*, separated on a silica gel column. Chromatograms show the Iatroscan response in μV *versus* the position along the 12 cm Chromarod following a partial scan. The arrow in (c) denotes the contamination of WE-II by WE-I due to incomplete column separation; HC = hydrocarbons.

a.




b.

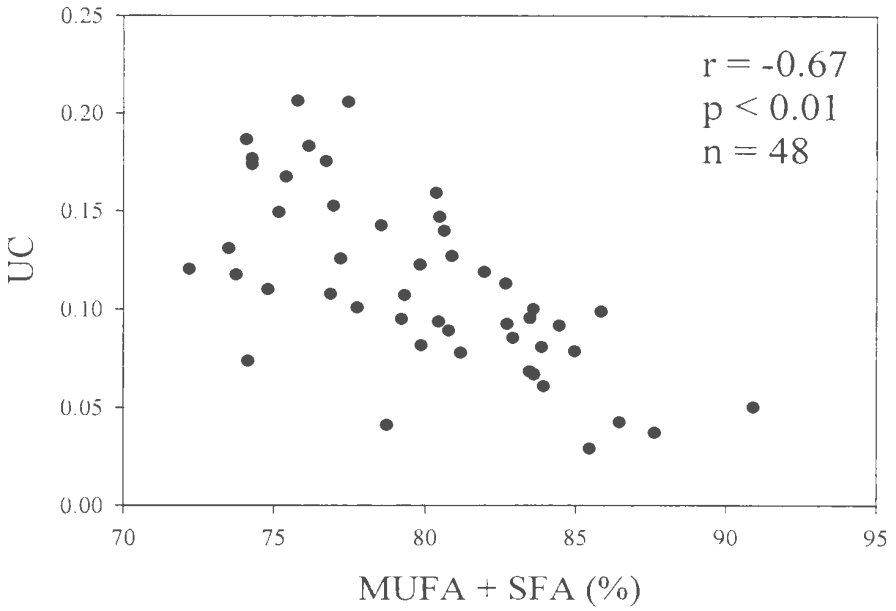



Figure 3.2. Degree of correlation (r=Pearson's product-moment correlation coefficient) between unsaturation coefficients (UC; Iatroscan-derived) and relative amounts of (a) polyunsaturated fatty acids (PUFA) and (b) monounsaturated (MUFA) + saturated (SFA) fatty acids (determined by gas chromatography (GC)) in experimental *Calanus glacialis* (CV).

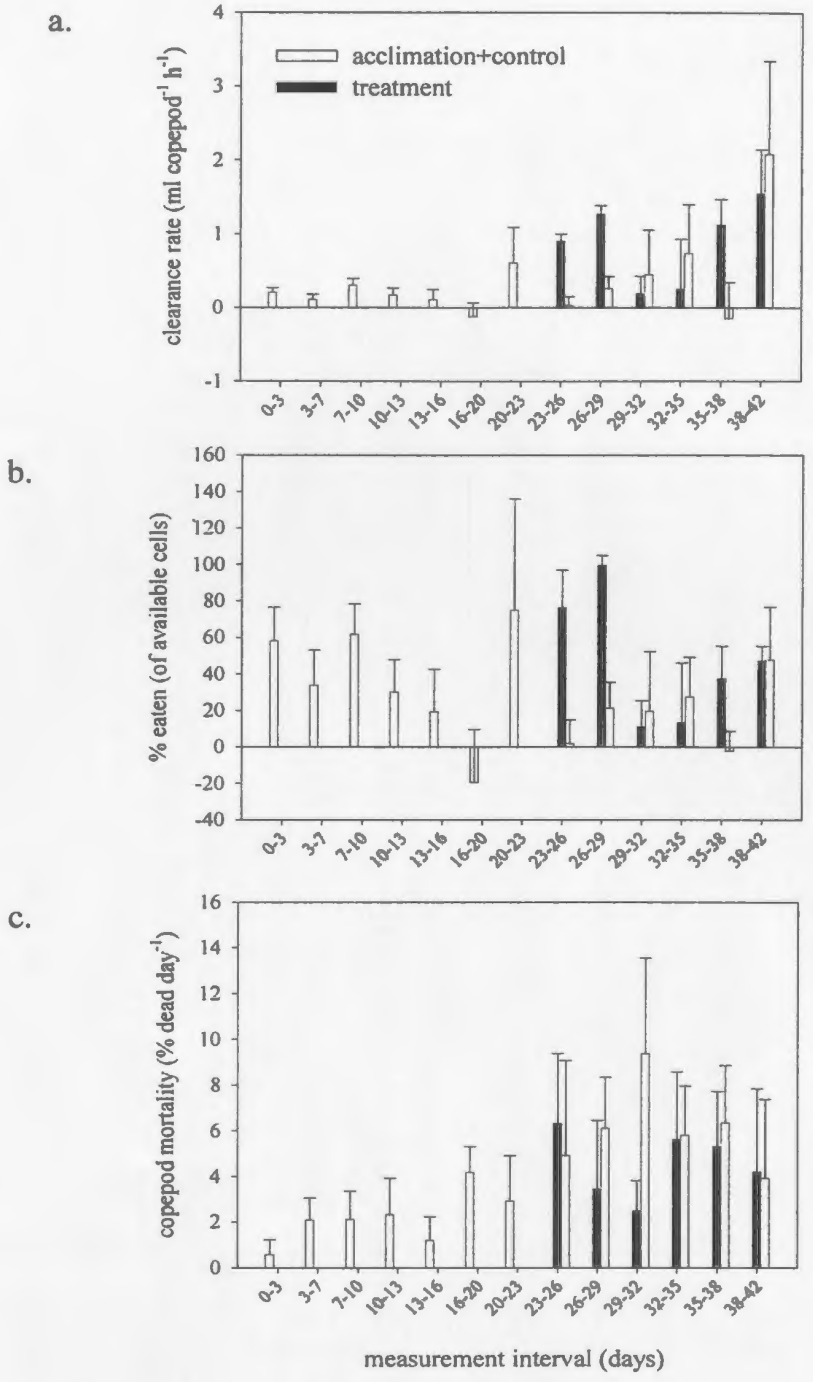



Figure 3.3. (a) Clearance rates (ml copepod$^{-1}$ h$^{-1}$), (b) percentage of food eaten (total cells eaten ÷ total cells provided at $t$=0), and (c) mortality rates (% dead day$^{-1}$) of *Calanus glacialis* (CV) during each 3-4 day measurement interval. Between days 0 and 23, all copepods were fed *Oxyrrhis marina* ('acclimation'); between days 23 and 42, 'treatment' copepods were fed *Thalassiosira hispida* and 'control' copepods, *O. marina* (see Methods). Error bars represent one standard deviation. Legend in (a) same for (b) and (c).

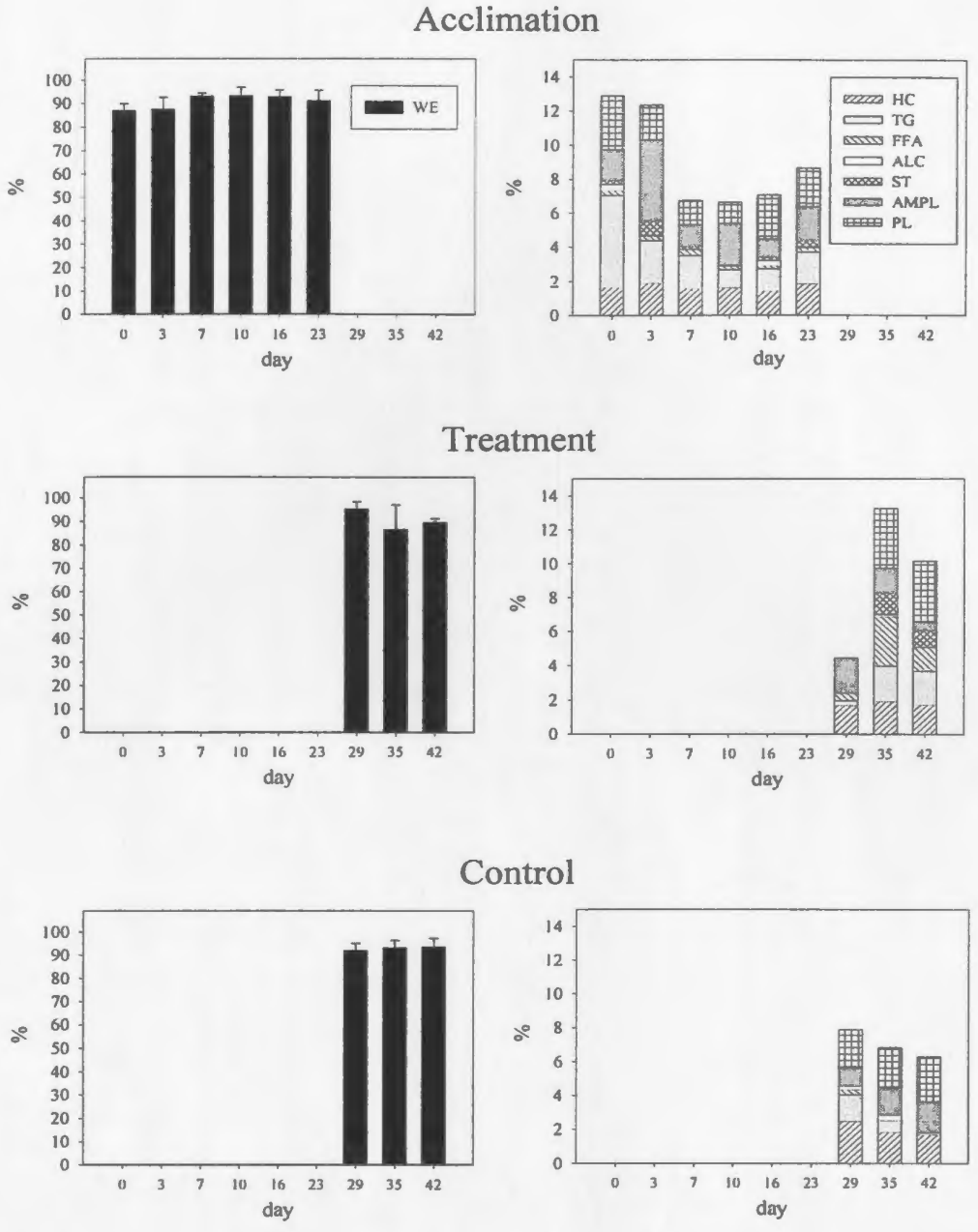



Figure 3.4. Relative lipid class composition (% of total lipid (sum of all lipid classes)) of *Calanus glacialis* (CV) where each lipid class bar represents a mean of 2-6 observations (± 1 standard deviation in the case of wax esters); WE=wax esters (left panels), HC=hydrocarbons, TG=triacylglycerols, FFA=free fatty acids, ALC=alcohols, ST=sterols, AMPL=acetone-mobile polar lipids, PL=phospholipids (right panels). Between days 0 and 23, all copepods were fed *Oxyrrhis marina* ('acclimation'; top panels); between days 23 and 42, 'treatment' (middle panels) copepods were fed *Thalassiosira hispida* and 'control' (bottom panels) copepods, *O. marina* (see Methods).

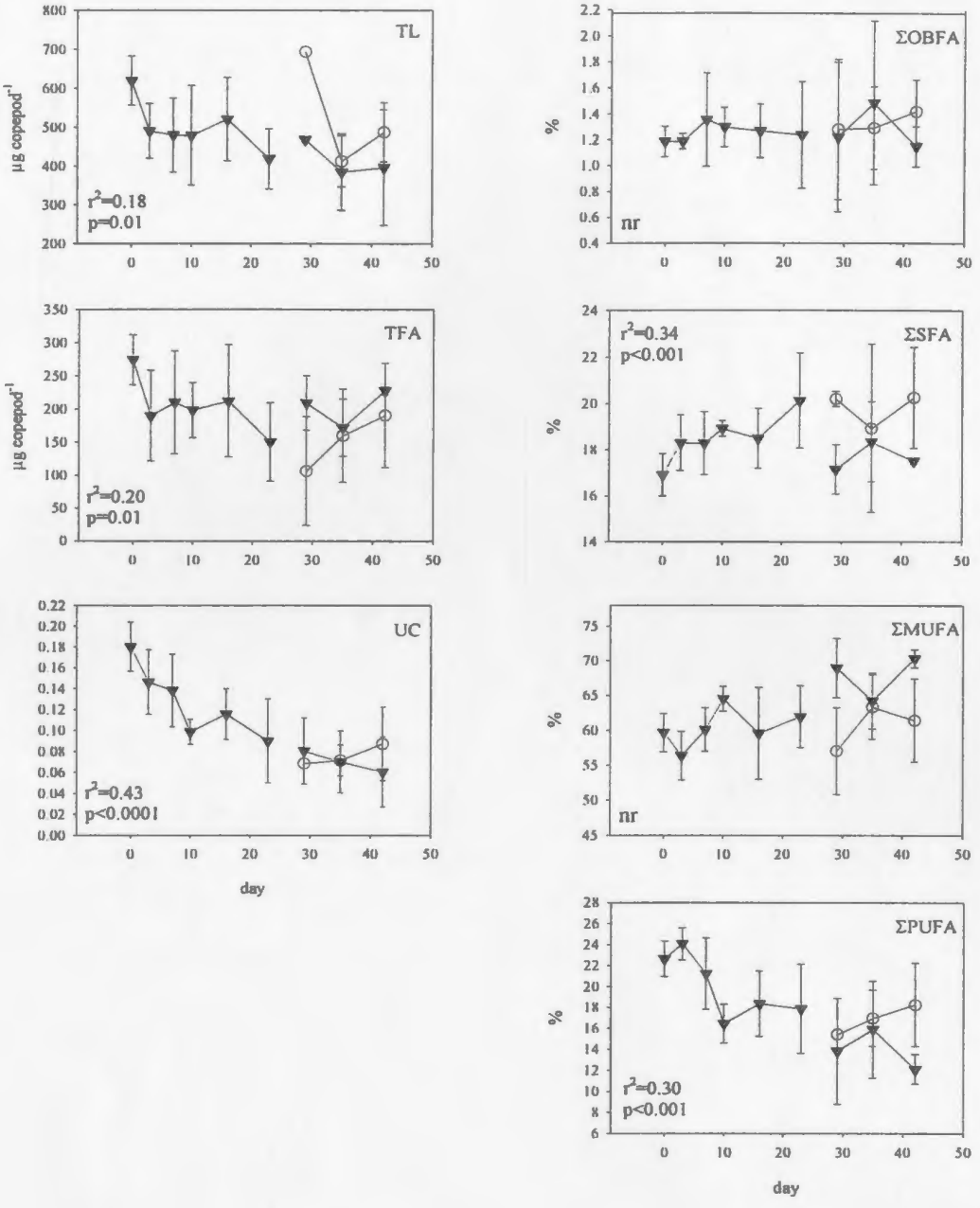



Figure 3.5. Relationships between mean total lipid (TL; µg copepod$^{-1}$), mean total fatty acids (TFA; µg copepod$^{-1}$), mean unsaturation coefficients (UC; dimensionless) and mean fatty acid groups (sum of percentages (% total fatty acids) of odd and/or branched (ΣOBFA), saturated (ΣSFA), monounsaturated (ΣMUFA) and polyunsaturated fatty acids (ΣPUFA)) and day in *Calanus glacialis* (CV) acclimation period (*Oxyrrhis marina*; triangles), treatment (*Thalassiosira hispida*; circles), and control (*O. marina*; triangles) samples. R-square and p-values represent results of model-I regression analyses of lipids in acclimation period samples (raw data) *versus* day (0-23) and 'nr' denotes no relationship.

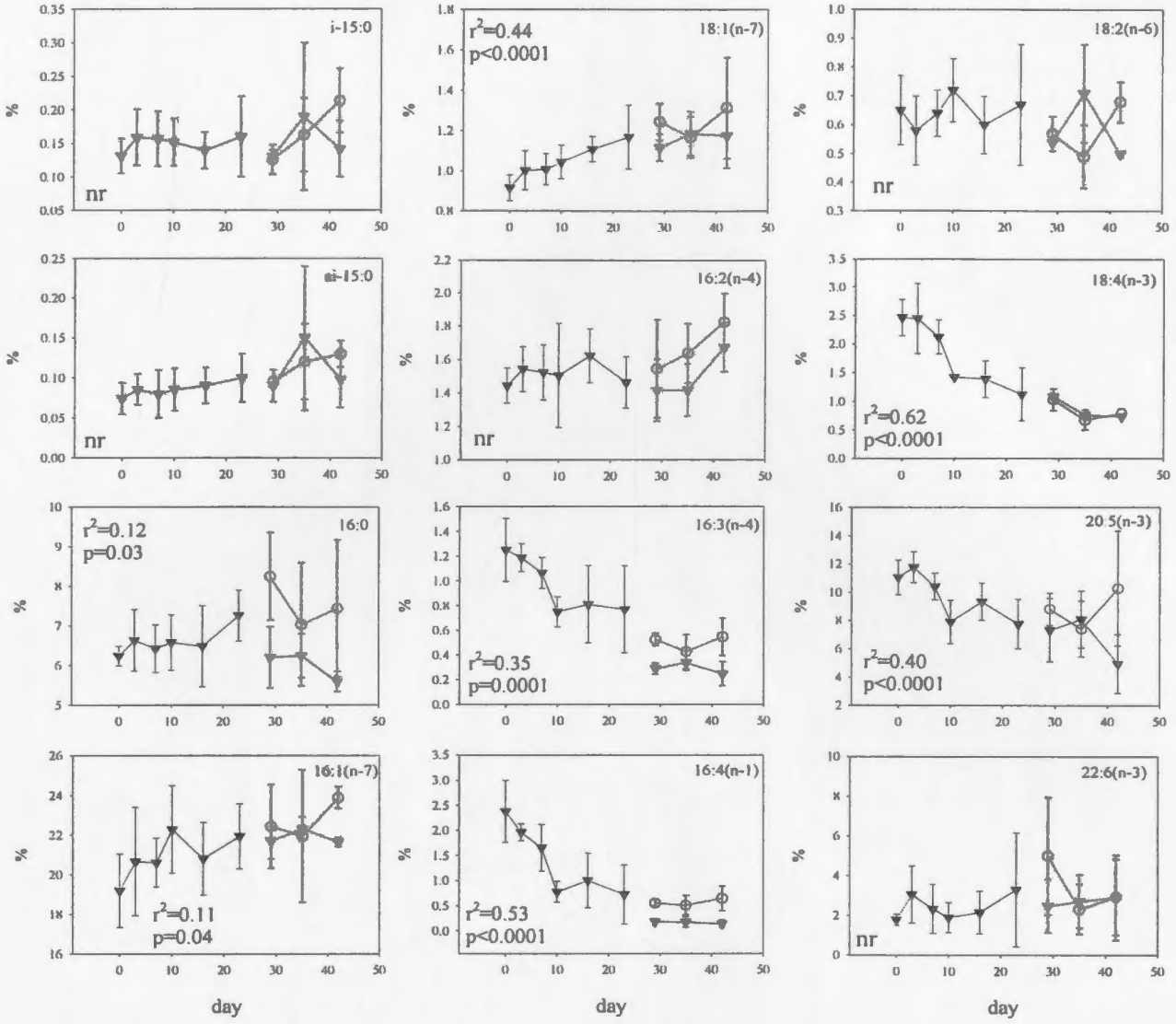
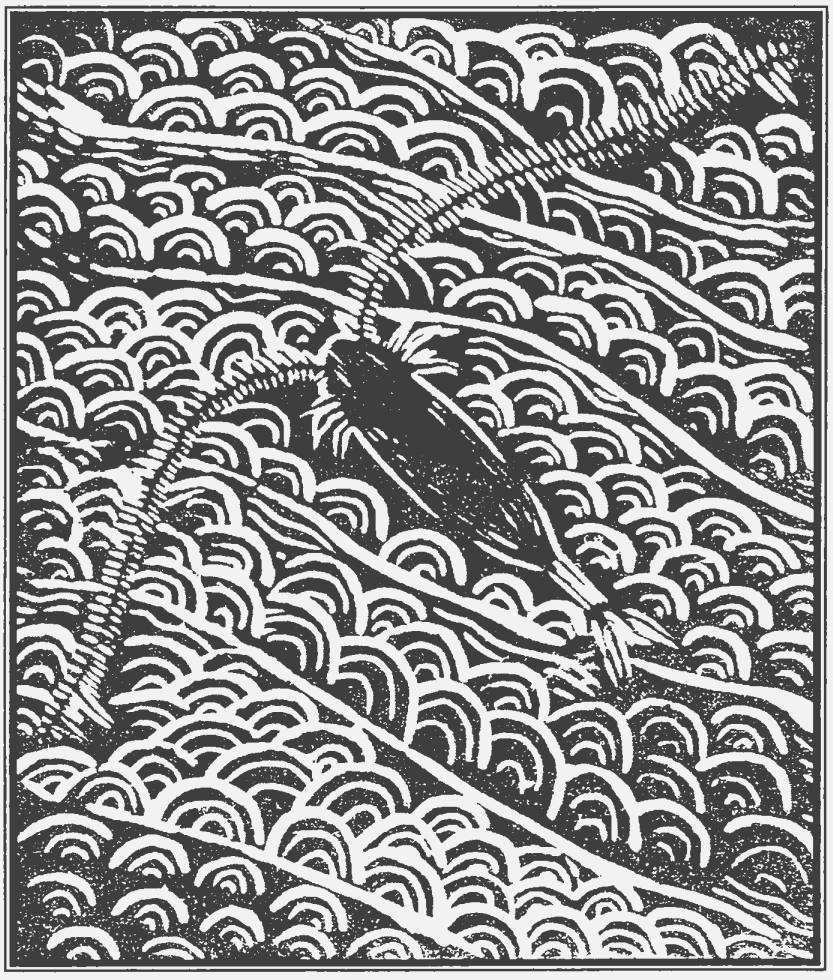



Figure 3.6. Relationships between mean percentages (% total fatty acids) of fatty acids specific to *Oxyrrhis marina* or *Thalassiosira hispida* (upper right corner of each graph; see Table 3.4) and day in *Calanus glacialis* (CV); organization of graphs as in Figure 3.5.

# Chapter 4. Species-specific differences in lipid composition and omnivory indices in arctic copepods collected in deep water during autumn (North Water Polynya)



## 4.1 Abstract

To discern species-specific patterns in omnivory indices in copepods from the North Water Polynya, lipid classes and fatty acids were determined in *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* females sampled below the mixed layer during autumn. Generally, *M. longa* contained higher proportions of triacylglycerols, polar lipids and 18:1(n-9) than the other species. *M. longa* also had lower relative amounts of wax esters (WE), polyunsaturated fatty acids (PUFA), 20:1(n-9), 22:1(n-11), and lower absolute (μg copepod$^{-1}$) and relative (% lipid) lipid levels. Unsaturation coefficients (UC; ratio of polyunsaturated to total WE) were usually lowest in *M. longa*. These differences probably relate to a reduced dependence on phytoplankton in *M. longa*, and hence more ingestion of PUFA-poor prey. Moreover, levels of the carnivory index 18:1(n-9)/18:1(n-7) were highest in *M. longa*. The data presented here support the widespread contention that *M. longa* is more omnivorous than *C. hyperboreus* and *C. glacialis*. Proportions of bacterial fatty acids (odd-numbered and/or branched (OBFA); 18:1(n-7)) and PUFA/SFA (SFA=saturated fatty acids) ratios were largely unrelated to feeding strategy. Relationships between relative and absolute amounts of 16:1(n-7) and 18:1(n-7) in copepods suggest that the latter fatty acid is formed *in vivo* by chain elongation of the former. However, elevated levels of 18:1(n-7) and OBFA in *M. longa* at stations dominated by the microbial loop imply that these indices can be used to track ingestion of PUFA-poor protozoans when diatoms are scarce.

## 4.2 Introduction

Omnivory is a stabilizing process in food webs: it serves to dampen oscillations between consumers and resources, thereby decreasing the likelihood of population extinction (McCann et al. 1998). In the marine environment, omnivory by copepods is common, especially during oligotrophic conditions when abundances of large phytoplankton are low relative to protozoans (Ohman and Runge 1994; Levinsen et al. 2000). Ingestion of protozoans may confer a reproductive advantage to copepods: they yield higher secondary production rates in mesocosms, compared to diatoms (Nejstgaard et al. 2001), and can contribute substantially to egg production *in situ* (White and Roman 1992). In some systems, protozoans are the primary grazers of phytoplankton (Gifford et al. 1995; Hansen et al. 1999). By feeding on this repackaged carbon, copepods represent an important link between the microbial food web and higher trophic levels (Gifford 1991).

The degree to which copepods are omnivorous is influenced by both morphology (Anraku and Omori 1963) and foraging tactics (Greene 1988). Generally, "carnivores" have spiny mouthparts and actively pursue their prey while "herbivores" rely on a feeding current and filter suspended cells with setose mouthparts. Despite these differences in behavior and morphology, copepods thought to be obligate filter feeders can switch to a raptorial feeding mode (Landry 1981; Gismervik and Anderson 1997) and copepods that usually feed raptorially can switch to filter feeding (Barnett 1974). Presumably this switching behavior occurs in response to changes in prey availability and

quality, but the mechanisms involved in selection are not understood. Given these potential sources of variability and the taxonomic diversity of copepod populations *in situ*, detailed species-specific dietary information is required to accurately predict carbon flow in marine food webs.

Lipid analysis of zooplankton provides much insight into diet history and feeding strategy. Fatty acids specific to diatoms, prymnesiophytes, dinoflagellates, ciliates, detritus, and metazoans have been used to infer ingestion of these foods (Mayzaud et al. 1976; Sargent et al. 1985; Kattner et al. 1989; Desvilettes et al. 1994; Ederington et al. 1995; Cripps and Hill 1998; Falk-Petersen et al. 1999). On this basis, and by using proportions of non-biomarker fatty acids and lipid classes (e.g., fatty alcohols, storage lipid, monounsaturated fatty acids, relative lipid content), copepods can be placed into broad ecological groups (i.e., omnivorous *versus* herbivorous; Falk-Petersen et al. 1987; Graeve et al. 1994a). While these findings are significant, omnivory indices capable of establishing *direct* links between zooplankton and important members of the microbial food web are required.

Levels of bacterial fatty acids (odd-numbered and/or branched (OBFA); (n-7) and (n-9) monounsaturates) have been used to demonstrate ingestion of microbial material (bacterivorous ciliates, sediment, free bacteria, detritus, symbiotic bacterial synthate) in a variety of invertebrate groups including crabs (Meziane and Tsuchiya 2000), gastropods (Pranal et al. 1996), bivalves (Zhukova et al. 1992), cladocerans (Desvilettes et al. 1994)

and polychaetes (Meziane et al. 1997). *In vitro* feeding experiments have shown that increases in proportions of OBFA (Ederington et al. 1995) and 18:1(n-7) and decreases in unsaturation coefficients (UC; ratio of polyunsaturated to total wax esters; Stevens et al. in press) occur in copepod tissues after ingestion of bacterivorous ciliates and flagellates. Low UC are believed to reflect ingestion of prey with a reduced polyunsaturated fatty acid (PUFA) content. The usefulness of these three omnivory indices in establishing connections between pelagic copepods and the microbial food web *in situ* has not been determined.

Similar lipid-based indices have been developed and used to demonstrate carnivory in other zooplankton groups. High ratios between PUFA and SFA (saturated fatty acids) signify carnivorous feeding in Antarctic krill (Cripps and Atkinson 2000). In addition, 18:1(n-9)/18:1(n-7) ratios have been used as carnivory indices in polar krill (Falk-Petersen et al. 2000), amphipods (Nelson et al. 2001; Auel et al. 2002), benthic invertebrates (e.g., decapods, echinoderms, polychaetes; Graeve et al. 1997) and deep-sea copepods (Auel 1999). These indices may also help deduce feeding strategies of pelagic copepods.

This work is part of the *International North Water Polynya Study*, a project aimed at determining food web patterns and evaluating the influence of physical parameters on biological processes. The North Water is a large area of open water (130 000 $km^2$), bounded by Ellesmere Island and Greenland, which is generated and maintained by local

winds and currents (Ingram et al. 2002). These physical conditions produce a spectrum of changing prey environments (Lovejoy et al. 2002) that present an ideal setting in which to investigate copepod omnivory. In this context, the usefulness of UC, 18:1(n-7) and OBFA in demonstrating connections between *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* females and the microbial food web during autumn was investigated. The utility of other dietary indices (i.e., PUFA/SFA, 18:1(n-9)/18:1(n-7)) was also evaluated. The chosen species are the most abundant of the large pelagic copepods found in the North Water (Ringuette et al. 2002) and are of central importance in oceanic food webs (Sargent et al. 1976). *Metridia* spp. and *Calanus* spp. occupy different ecological niches (references in Norrbin et al. 1990) and it is hypothesized that the proposed omnivory indices, as well broad lipid data, will reflect their respective feeding strategies. This study represents the first comprehensive analysis of zooplankton lipids in the North Water.

## 4.3 Materials and Methods

### *4.3.1 Classification of regions in the North Water*

Sampled stations were grouped into geographical regions according to Lovejoy et al. (2002) who found distinct spatial trends in microplankton assemblages in the North Water in July 1998 (Figure 4.1). Although the copepod lipid data presented here were collected in autumn (September-October 1999), there is a considerable time lag in biomarker accumulation after prey ingestion (ca. 6 weeks; Graeve et al. 1994b), making late summer protist distributions particularly relevant. Given that the phytoplankton

bloom was unusually late at Station 58 and microbial processes dominated until September (Booth et al. 2002; Lovejoy et al. 2002), this station was not grouped with others in the region (i.e., Station 54 near the Carey Islands; Figure 4.1).

*4.3.2 Field sampling*

From 1 September to 1 October 1999, discrete strata were sampled below the mixed layer at 11 stations in the North Water using a messenger-activated closing net system equipped with 200 μm mesh nets and partially closed codends (Table 4.1; Figure 4.1). All nets were towed vertically. Sampled strata represented low chlorophyll environments; all were below both the thermocline and sub-surface chlorophyll maximum, as verified by *in situ* fluorescence and CTD (conductivity, temperature, depth) traces. Nets were retrieved at a speed of 0.3-0.5 m $s^{-1}$. As soon as the nets came on board, the contents of the codends were gently placed in coolers containing surface seawater.

Single or triplicate samples of *Calanus hyperboreus* CV (2-6 individual $sample^{-1}$), *C. glacialis* CV (4-10 ind. $sample^{-1}$) and *Metridia longa* females (5-27 ind. $sample^{-1}$) were picked out of the net tow catches using the blunt end of a pipette, a dissecting microscope, and a 10 ml sorting cell (Table 4.1). Sorted copepods were placed in specimen cups filled with ~50 ml filtered seawater (0.2 μm) and kept on ice. After a sufficient number was picked, the contents of the cups were filtered onto combusted 25 mm GF/C filters and then folded, quick-frozen on an aluminum block (pre-cooled to -80°C), placed in combusted foil envelopes, and stored at -80°C. Unsorted assemblages

of copepods (~200-300 individuals) were also taken from each net tow, collected on combusted 47 mm GF/C filters, quick-frozen, and stored as above. Later, filters were partially thawed and copepods were removed and sorted for dry mass analysis and isolation of wax ester (see below).

*4.3.3 Dry mass analysis*

Between 1 and 5 *C. hyperboreus* CV, 1-8 *C. glacialis* CV and 1-13 *M. longa* females were gently removed from partially frozen filters and placed in pre-weighed, combusted tin cups. Where possible (~70% of the time), 1-2 additional replicate samples were taken. The tin cups were then dried at ~60°C and weighed on a Mettler Toledo microbalance (UMT2).

*4.3.4 Wax ester isolation*

A total of 48 *Calanus hyperboreus* (females, CV, CIV, CIII) were removed from filters collected at Station 6, placed in 2 ml chloroform and total lipids extracted (see below). Following Ohman (1997), lipids were dried, re-suspended in hexane, and applied to a combusted glass column with a 6 ml bed volume of silica gel. Wax esters (WE) were eluted with 45 ml 1% diethyl ether in hexane, dried under a stream of $N_2$, and weighed to constant mass. This native WE (95% pure) was then used to calibrate the Iatroscan. Stevens et al. (in press) found that during Iatroscan development, WE in *C. hyperboreus* split according to the degree of unsaturation of acyl lipid. The first peak contained predominantly saturated and monounsaturated fatty acids, whereas the second contained

mostly polyunsaturated fatty acids. Unsaturation coefficients (UC: Stevens et al. in press) were calculated as the ratio of the second WE peak area to that of total WE (peak 1 + peak 2).

*4.3.5 Determination of lipid classes and fatty acids*

Frozen samples of sorted copepods were brought to the laboratory on ice and placed in 2 ml chloroform. Lipids were extracted following Parrish (1999). To separate and quantify lipid classes, samples were manually spotted on silica-coated Chromarods (SIII), developed, and passed through the flame ionization detector (FID) of an Iatroscan MK V. The air and hydrogen flow rates were set to 2 l $min^{-1}$ and 190 ml $min^{-1}$, respectively. Rod development was done following Parrish (1987) where WE were resolved by double development in a non-polar solvent system (hexane:diethyl ether:formic acid (99:1:0.05), 25 min + 20 min). Commercial standards (Sigma-Aldrich Canada Ltd.) were used to calibrate the Iatroscan and establish peak identities.

Fatty acids were quantified as methyl esters by FID using a Varian 3400 gas chromatograph (GC), following total lipid derivatization of samples with $BF_3$-methanol (85°C, 1 h). Methyl esters were analyzed on an Omegawax column following Budge and Parrish (1998). Tricosanoic acid (23:0) was used as an internal standard at a concentration of ~10% total fatty acids. Peaks were identified by comparing sample retention times to those of commercial standard mixtures (Supelco, Sigma-Aldrich Canada Ltd.) following Ackman (1986), and by using a Varian 2000 GC/mass

spectrometer. The term "odd and/or branched fatty acids" (OBFA) is used to describe those fatty acids that have odd-numbered carbon chains (with the exception of 21:5(n-3)) and/or iso or anteiso branches.

*4.3.6 Statistical analysis*

To test for species-specific differences in omnivory indices between *Calanus hyperboreus*, *C. glacialis* and *Metridia longa*, a one-way ANOVA was performed for each region of the polynya, in conjunction with post-hoc multiple comparisons (Tukey; SPSS 9.0). All testing was done on untransformed values. Model-I regressions were used to describe relationships between 16:1(n-7) and 18:1(n-7) in copepod tissues (SPSS 9.0). For all statistical testing, the rejection criterion was set to $\alpha$=0.05.

## 4.4 Results

Across all regions, *Calanus hyperboreus* CV dry mass ranged from 2.4 to 3.6 mg (Table 4.2). *C. glacialis* CV (1.0-1.4 mg) and *Metridia longa* females (0.4-0.5 mg), weighed considerably less. The total lipid content of *C. hyperboreus* (1.5-1.8 mg ind.$^{-1}$) was 3-4 times greater than that of *C. glacialis* (0.4-0.7 mg ind.$^{-1}$) and 10-37 times higher than in *M. longa* (0.04-0.2 mg ind.$^{-1}$). On a dry mass basis, *C. hyperboreus* and *C. glacialis* were 46-70% and 32-58% lipid, respectively, while *M. longa* contained 11-35% lipid. Within species, regional differences in lipid class composition and lipid content were minor in *C. hyperboreus* and *C. glacialis*. However, *M. longa* from the Carey Island

(CI) region and Station 58 had much lower total lipid levels and contained proportionally less lipid as a function of body mass than in the other regions.

Wax esters (WE) were the major lipid class in all species, ranging between 49 and 93% of total lipid (Table 4.2). Generally, *Calanus hyperboreus* contained higher relative amounts of WE (84-93%) than either *C. glacialis* (82-90%) or *Metridia longa* (49-75%); the latter species always had the lowest values. Triacylglycerols (TG; Smith Sound (SS), Central Northern Baffin Bay (CNBB)) or phospholipids (PL; CI, West Greenland Current (WGC), Station 58) were the next most abundant lipid classes. Proportions of TG were generally lower in *C. hyperboreus* (0-6%) than in *C. glacialis* (5-9%) and *M. longa* (5-13%) whose values were roughly equal. The relative abundance of PL was always highest in *M. longa* (6-38%) and lowest in *C. hyperboreus* (2-5%) and *C. glacialis* (2-3%). Hydrocarbons (HC; 0-3% across all samples) and acetone-mobile polar lipids (AMPL; 1-5%) were less important lipid classes. Proportions of methyl esters (ME), free fatty acids (FFA), alcohols (ALC), sterols (ST), and diacylglycerols (DG) were almost always < 1% of total lipid (data not shown). *M. longa* consistently had higher proportions of polar (AMPL, PL) lipid classes (10-43%) than either *Calanus* species (3-7%), which were richer in neutral lipid (i.e., WE, TG, ME, FFA, ALC, ST, DG).

In all but two cases (*Metridia longa* in WGC and Station 58), 16:1(n-7) and 20:5(n-3) were the two most abundant fatty acids in *Calanus hyperboreus*, *C. glacialis* and *M. longa* (16:1(n-7)=15-30%, 20:5(n-3)=11-30% total fatty acids overall; Table 4.3).

*M. longa* was somewhat different from the two *Calanus* species in terms of its fatty acid composition. It contained relatively less 20:1(n-9) and 22:1(n-11), and more 18:1(n-9) and 24:1. Moreover, *M. longa* had lower relative amounts of 16:4(n-1) and 20:5(n-3), and more 22:6(n-3). In the WGC region, 22:6(n-3) was particularly abundant in *M. longa*, accounting for 19% of the total fatty acids. In regions SS, CNBB and CI, proportions of 16:1(n-7) in *M. longa* were comparable to or greater than in *C. hyperboreus* and *C. glacialis*. Except for the WGC region, *M. longa* contained lower proportions of polyunsaturated fatty acids (PUFA) and larger relative amounts of monounsaturated fatty acids (MUFA) than the other two species. All species contained large amounts of (n-3) PUFA and small amounts of (n-6) PUFA. The major difference between the two *Calanus* species was that *C. glacialis* had higher proportions of saturated fatty acids (SFA), principally 14:0.

In the SS, CNBB and CI regions, *Metridia longa* had significantly lower mean unsaturation coefficients (UC) than both *Calanus* species (SS: $p<0.001$, CNBB: $p<0.01$, CI: $p<0.04$: Figure 4.2a). At Station 58, UC was notably low in *M. longa* (0.03) and appeared somewhat reduced in *C. hyperboreus* (0.30) and *C. glacialis* (0.26). In the WGC region, mean UC were equivalent across species ($p=0.521$-$0.921$). The proportion of odd and/or branched fatty acids (OBFA) was usually less than 2% of the total (Figure 4.2b). In the SS and CNBB regions of the polynya, *C. glacialis* had significantly higher OBFA levels than *C. hyperboreus* (SS: $p<0.02$, CNBB: $p<0.04$). No other species-specific differences were found in these or other regions. At Station 58, unusually high

and moderately elevated OBFA levels were observed in *M. longa* (3.9%) and *C. hyperboreus* (1.6%), respectively.

In regions SS and CNBB, relative proportions of 18:1(n-7) were highest in *Metridia longa* followed by *Calanus hyperboreus* and *C. glacialis* (SS: $p \leq 0.04$, CNBB: $p < 0.01$; Figure 4.2c). In regions CI and WGC, and at Station 58, the same trend was observed, but the patterns of statistical significance were different. In the CI region, a difference in the proportion of 18:1(n-7) was observed only between *M. longa* and *C. glacialis* ($p < 0.03$), while in the WGC region, *C. glacialis* had lower levels than the other two species ($p < 0.01$). At Station 58, relative 18:1(n-7) levels were elevated in *M. longa* (3.2%) and *C. hyperboreus* (1.8%). Moderate to strong relationships were found between relative ($r^2 = 0.34$, $p < 0.001$; Figure 4.3a) and absolute ($r^2 = 0.55$, $p < 0.001$; Figure 4.3b) amounts of 18:1(n-7) and 16:1(n-7) in copepod tissues.

In all regions, PUFA/SFA ratios were generally highest in *Calanus hyperboreus*, followed by *Metridia longa* and *C. glacialis* (Figure 4.2d). These species-specific differences were statistically significant only in the SS region of the polynya ($p < 0.01$). In the CI region, PUFA/SFA ratios were significantly higher in *C. hyperboreus*, relative to the two other species ($p \leq 0.04$). In the CNBB and WGC regions, PUFA/SFA ratios were significantly higher in *C. hyperboreus*, as compared to levels in *C. glacialis* (CNBB: $p < 0.02$, WGC: $p < 0.03$). At Station 58, PUFA/SFA ratios were reduced in all copepod species (*C. hyperboreus*=4.0, *C. glacialis*=2.2, *M. longa*=1.7). The 18:1(n-9)/18:1(n-7)

ratio was highest in *M. longa*, in all regions of the polynya, except at Station 58 (Figure 4.2e; SS: $p<0.001$, CNBB: $p<0.001$, CI: $p<0.01$, WGC: $p<0.02$). In the SS, CNBB and WGC regions, 18:1(n-9)/18:1(n-7) ratios were also significantly higher in *C. glacialis*, relative to *C. hyperboreus* (SS: $p<0.01$, CNBB: $p<0.01$, WGC: $p<0.04$). Values of this ratio were notably low in *M. longa* at Station 58 (3.9).

## 4.5 Discussion

As a result of thick first-year ice cover on the southern border of the North Water in 1998, sampling of Station 58 was not possible until mid-June (Lovejoy et al. 2002). There is thus an incomplete picture of microplankton dynamics at this station, compared to others where a semi-continuous 6-month period of data exists (August 1997, April-July 1998, August-October 1999). From the available data, it appears that substantial accumulations of diatoms (mainly *Chaetoceros socialis*) did not occur until September at Station 58, while the major phytoplankton bloom at nearby Station 54 (mostly *Thalassiosira* and *Fragilariopsis* spp.) occurred in May (Booth et al. 2002). Therefore, although both stations were characterized by large numbers of microbial prey (dinoflagellates, ciliates, small flagellates) and few diatoms in July (Lovejoy et al. 2002), the copepods at Station 58 were likely in a pre- and not a post-bloom state. Also, Middleboe et al. (2002) found that bacterial abundance and production rates were much higher at Station 58 than at Station 54 in July, indicating the enhanced importance of the microbial loop. Although Station 58 was included in the Carey Island region by Lovejoy et al. (2002), it was not labeled as such here because it represented a unique case.

The lipid compositions of all three copepod species, particularly the high levels of 16:1(n-7) and 20:5(n-3), indicate that diatoms were an important food source in September in the polynya. Diatom production is prolonged in the North Water, with a sizeable *Chaetoceros socialis* population persisting well into autumn (Booth et al. 2002). Dinoflagellate biomarker (22:6(n-3), 18:4(n-3)) levels were high in copepods from the southeastern polynya, particularly in *Metridia longa* females from the WGC region where microbially-based microplankton (dinoflagellates, ciliates, small flagellates) dominated at the 1% light depth in July (Lovejoy et al. 2002). The fatty acid 16:4(n-1) is a particularly useful diatom biomarker (Viso and Marty 1993). Higher proportions of this fatty acid in the two *Calanus* species, relative to *M. longa*, suggest that the former were more dependent on diatoms. During periods of food scarcity, arctic copepods preferentially conserve energetic long-chain monounsaturated fatty acids and use stored PUFA to fuel metabolism (Lee 1974). Despite the lateness of the season, the high PUFA contents and relatively low 20:1(n-9) and 22:1(n-11) levels suggest ongoing active feeding during the sampling period. Furthermore, phytoplankton pigments were detected in copepod guts at several stations in the polynya in autumn (H. Hattori et al. unpublished data).

Arctic and Antarctic copepods, including *Metridia* spp. and *Calanus* spp., have distinct lipid profiles relating to feeding strategy. Relative to carnivorous or highly omnivorous species, copepods that rely heavily on phytoplankton store mostly WE and very little TG, contain higher proportions of 20:1(n-9), 22:1(n-11) and PUFA, have longer-chain fatty alcohols in their wax esters, depend highly on lipid stores, and are

characterized by a strong seasonal accumulation of lipid (Falk-Petersen et al. 1987; Graeve et al. 1994a; Albers et al. 1996). Although fatty alcohols were not analyzed, nor a seasonal study performed, in all other respects the data presented here confirm that *C. hyperboreus* CV and *C. glacialis* CV were feeding more herbivorously than were *M. longa* females.

The unsaturation coefficients corroborate this conclusion. The higher UC noted in *Calanus hyperboreus* and *C. glacialis* indicate that these species ate proportionally more phytoplankton than *Metridia longa*. Although *Metridia* spp. eat diatoms and autotrophic dinoflagellates, known to be rich in PUFA (Viso and Marty 1993), their diet can also include substantial amounts of heterotrophic prey including ciliates, flagellates, detritus and the small omnivorous copepods *Oithona* spp. and *Oncaea* spp. (reviewed in Romano et al. 1999 for *M. gerlachei*). Lipid data on these prey items are scarce. However, bacterivorous marine ciliates (Harvey et al. 1997) and *Oithona* spp. from the marginal ice zone of the Bellingshausen Sea (Cripps and Hill 1998) contain only small proportions of PUFA. Large pelagic copepods incorporate dietary fatty acids relatively unchanged into storage lipid (Lee et al. 1971). UC values thus mirror the relative PUFA content of the prey. This index is determined during routine Iatroscan analysis. Given the robustness of this system compared to other methods (reviewed in Parrish et al. 2000), the resulting estimates of copepod feeding strategy are more accurate, replicable and quickly obtained.

UC and PUFA/SFA were not in agreement. It was expected that *M. longa* would have the lowest values of the PUFA/SFA ratio, relative to both *Calanus* species, since it is known to store less PUFA (Falk-Petersen et al. 1987; Albers et al. 1996). Instead, PUFA/SFA ratios in *M. longa* were generally greater than or equivalent to levels in *C. glacialis* and *C. hyperboreus*, respectively. This may relate to the high phospholipid (PL) content of *M. longa* (see Table 4.2), as compared to the two *Calanus* species. Since PUFA/SFA ratios are usually calculated after total lipid derivatization (e.g., Cripps and Atkinson 2000; Auel et al. 2002), they contain significant amounts of structural PUFA. Calanoid copepod PL is rich in PUFA, primarily 20:5(n-3) and 22:6(n-3) (Fraser et al. 1989; Albers et al. 1996; Scott et al. 2002). PUFA/SFA ratios may therefore be inappropriate for species-specific comparisons when the animals of interest differ in their relative PL content. The PUFA/SFA index was not useful in identifying species-specific and regional differences in Arctic hyperiid amphipod feeding strategy (Auel et al. 2002). As mirrors of ingested prey, UC provided more accurate estimates of feeding strategy in copepods from the polynya than did PUFA/SFA ratios.

At Station 58, UC values were low in all three species, particularly *M. longa*, relative to other regions in the North Water. This result was not surprising, given that until September copepods from this station were probably exposed mostly to microbial prey (see references above). It therefore appears that UC are also sensitive to spatial changes in prey composition. The low values at Station 58 are presumably directly related to a sustained period of microbial activity (low availability of PUFA-rich

phytoplankton). During post-bloom conditions, when diatoms are less dominant, *Calanus* spp. and *Metridia* spp. from the North Water ingest substantial numbers of protozoans (Saunders et al. unpublished data). No species-specific differences in UC were detected in the WGC region where all species, especially *M. longa*, were probably consuming significant amounts of PUFA-rich dinoflagellates.

Proportions of odd and/or branched fatty acids (OBFA) in copepod tissues did not relate to feeding strategy. There is little reason to believe that OBFA levels should be higher in *Calanus glacialis* than in *C. hyperboreus* in the SS and CNBB regions, especially since *M. longa* values were equivalent to those in both of these species. At Station 58, however, *M. longa* had elevated OBFA levels, relative to the other species and regions, which agrees with the UC data. Perhaps OBFA accumulate in copepod tissues only after prolonged feeding on prey high in these compounds and such conditions are rare in the diatom-driven North Water. Although detailed information on protozoan assemblages at Station 58 is lacking for September, the copepod fatty acid profiles observed here could reflect ingestion of bacterivorous microzooplankton.

At first glance, it appears that levels of the bacterial fatty acid 18:1(n-7) in copepod tissues mirror differences in feeding strategy, since relative amounts were significantly higher in *Metridia longa* than in one or both *Calanus* species in most regions of the North Water. However, differences between *C. hyperboreus* and *C. glacialis* were also significant three-quarters of the time. Close inspection of the fatty

acid data revealed that proportions of 16:1(n-7) were often higher in *M. longa* and *C. hyperboreus* than in *C. glacialis* (Table 4.3). Furthermore, there were moderate to strong relationships between relative and absolute amounts of 16:1(n-7) and 18:1(n-7) in copepod tissues. It is possible that 18:1(n-7) levels in all species are a direct result of *in vivo* chain elongation of dietary 16:1(n-7) (Kattner and Hagen 1995). On the other hand, proportions of 18:1(n-7) in *M. longa* were elevated at Station 58, relative to other regions, and this fatty acid (but not 16:1(n-7)) was shown to increase in *C. glacialis* following a 6-week diet of bacterivorous *Oxyrrhis marina* (*O. marina*: 15% 18:1(n-7), 16:1(n-7)/18:1(n-7)=0.1; Stevens et al. in press). Although this finding suggests that 18:1(n-7) is a promising omnivory index, results could easily be misinterpreted if elongation is an important process and, perhaps, where 16:1(n-7) to 18:1(n-7) ratios are high in the prey.

High values of the 18:1(n-9)/18:1(n-7) omnivory index in *M. longa*, relative to the two *Calanus* species, indicated that during fall in the polynya, this species was the most carnivorous. The data also suggest that *C. glacialis* was generally feeding more carnivorously than *C. hyperboreus*. These patterns conformed to the other lipid data and the 18:1(n-9)/18:1(n-7) ratio may therefore be a useful omnivory index for pelagic calanoids. The underlying assumption of this index is that 18:1(n-9) is derived directly from animal prey, while 18:1(n-7) is formed *in vivo* by chain elongation of the diatom fatty acid, 16:1(n-7) (e.g., Graeve et al. 1997; Falk-Petersen et al. 2000). In cases where diatom productivity is high, as it was in much of current sampling period, the

18:1(n-9)/18:1(n-7) index will probably work well. However, in oligotrophic conditions, this index may be complicated by bacterial input in the diet. Bacteria produce significant amounts of (n-7) and (n-9) monounsaturates, including 18:1(n-7) (Sargent et al. 1987; Pranal et al. 1996 and references therein). Graeve et al. (1997) observed markedly low 18:1(n-9)/18:1(n-7) ratios among deposit-feeding brittle stars. Although the authors asserted that the high 18:1(n-7) content derived from chain elongation of dietary 16:1(n-7), a high bacterial input is an equally likely scenario in the benthic environment. Low values of the 18:1(n-9)/18:1(n-7) ratio in *M. longa* at Station 58 may be an example of this problem, since microbial prey were probably an important part of the diet (high OBFA and 18:1(n-7)).

## 4.6 Conclusions

Most omnivory indices tested in this chapter could be used to demonstrate connections between copepods and the microbial food web. Unsaturation coefficients were capable of distinguishing between arctic copepods on the basis of feeding strategy and levels in animals were also related to spatial differences in prey assemblages. An alternative index, PUFA/SFA, was inappropriate for species-specific comparisons because the copepods of interest differed in their relative phospholipid content. Proportions of bacterial fatty acids (OBFA, 18:1(n-7)) were less sensitive to differences in feeding strategy perhaps because much biological production in the North Water is tied to intense and prolonged diatom blooms. Bacterial fatty acids may be particularly useful during oligotrophic conditions and in more opportunistic copepod species.

18:1(n-9)/18:1(n-7) ratios proved useful for determining copepod feeding strategy; however, this index may not be appropriate when bacteria are an important component in the diet. The omnivory indices explored here may aid in predicting carbon flow via copepods as further investigations into the North Water food web continue.

## 4.7 Acknowledgements

The author would like to thank the officers, crew and captain of the *N.G.C.C. Pierre Radisson*, as well as M. Fortier, N. Kobayashi, M. Ringuette, M. Sampei, P. Sirois and K. Takahashi for assistance in the field. Funding for this project was provided by an NSERC grant awarded to D. Deibel, R. Rivkin and M. Anderson, the NSERC Discovery Grant of D. Deibel, and a Northern Scientific Training Program grant awarded to C. Stevens.

## 4.8 References


Ackman RG (1986) WCOT (Capillary) Gas-liquid chromatography. In: Hamilton RJ, Rossell JB (eds) Analysis of Oils and Fats. Elsevier, London, pp 137-206

Albers CS, Kattner G, Hagen W (1996) The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptations. Mar Chem 55:347-358

Anraku M, Omori M (1963) Preliminary survey of the relationship between the feeding habit and the structure on the mouth-parts of marine copepods. Limnol Oceanogr 8:116-126

Auel H (1999) The ecology of Arctic deep-sea copepods (Euchaetidae and Aetideidae). Aspects of their distribution, trophodynamics and effect on the carbon flux. Ber Polarforsch 319:1-97

Auel H, Harjes M, de Rocha R, Stübing D, Hagen W (2002) Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellulua*. Polar Biol 25:374-383

Barnett AM (1974) The feeding ecology of an omnivorous neritic copepod, *Labidocera trispinosa* Esterly. Ph. D. thesis, University of California, San Diego

Booth BC, Larouche P, Bélanger S, Klein B, Amiel D, Mei Z-P (2002) Dynamics of *Chaetoceros socialis* blooms in the North Water. Deep-Sea Res II 49:5003-5025

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29:1547-1559

Cripps GC, Hill HJ (1998) Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. Deep-Sea Res I 45:1357-1381

Cripps GC, Atkinson A (2000) Fatty acid composition as an indicator of carnivory in Antarctic krill, *Euphausia superba*. Can J Fish Aquat Sci 57:31-37

Desvilettes Ch, Bourdier G, Breton JC, Combrouze Ph (1994) Fatty acids as organic markers for the study of trophic relationships in littoral cladoceran communities. J Plankton Res 16:643-659

Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. Limnol Oceanogr 40:860-867

Falk-Petersen S, Sargent JR, Tande KS (1987) Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol 8:115-120

Falk-Petersen S, Sargent JR, Lønne OJ, Timofeev S (1999) Functional biodiversity of lipids in Antarctic zooplankton: *Calanoides acutus*, *Calanus propinquus*, *Thysanoessa macrura* and *Euphausia crystallorophias*. Polar Biol 21:37-47

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57:178-191

Fraser AJ, Sargent JR, Gamble JC (1989) Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis*

Müller from a nutrient-enriched seawater enclosure. J Exp Mar Biol Ecol 130:81-92

Gifford DJ (1991) The protozoan-metazoan trophic link in pelagic ecosystems. J Protozool 38:81-86

Gifford DJ, Fessenden LM, Garrahan PR, Martin E (1995) Grazing by microzooplankton and mesozooplankton in the high-latitude North Atlantic Ocean: spring *versus* summer dynamics. J Geophys Res 100:6665-6675

Gismervik I, Anderson T (1997) Prey switching by *Acartia clausi*: experimental evidence and implications of intraguild predation assessed by a model. Mar Ecol Prog Ser 157:247-259

Graeve M, Hagen W, Kattner G (1994a) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep-Sea Res I 41:915-924

Graeve M, Kattner G, Hagen W (1994b) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J Exp Mar Biol Ecol 182:97-110

Graeve M, Kattner G, Piepenburg D (1997) Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biol 18:53-61

Greene CH (1988) Foraging tactics and prey-selection patterns of omnivorous and carnivorous calanoid copepods. Hydrobiologia 167/168:295-302

Hansen BW, Nielsen TG, Levinsen H (1999) Plankton community structure and carbon cycling on the western coast of Greenland during the stratified summer situation. III. Mesozooplankton. Aquat Microb Ecol 16:233-249

Harvey HR, Ederington MC, McManus GB (1997) Lipid composition of the marine ciliates *Pleuronema* sp. and *Fabrea salina*: shifts in response to changes in diet. J Eukaryot Microbiol 44:189-193

Ingram RG, Bâcle J, Barber DG, Gratton Y, Melling H (2002) An overview of physical processes in the North Water. Deep Sea Res II 49:4893-4906

Kattner G, Hirche HJ, Krause M (1989) Spatial variability in lipid composition of calanoid copepods from Fram Strait, the Arctic. Mar Biol 102:473-480

Kattner G, Hagen W (1995) Polar herbivorous copepods - different pathways in lipid biosynthesis. ICES J Mar Sci 52:329-335

Landry MR (1981) Switching between herbivory and carnivory by the planktonic marine copepod Calanus pacificus. Mar Biol 65:77-82

Lee RF, Nevenzel JC, Paffenhöfer G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9:99-108

Lee RF (1974) Lipid composition of the copepod *Calanus hyperboreus* from the Arctic Ocean. Changes with depth and season. Mar Biol 26:313-318

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204:65-77

Lovejoy C, Legendre L, Martineau M-J, Bâcle J, von Quillfeldt CH (2002) Distribution of phytoplankton and other protists in the North Water. Deep-Sea Res II 49:5027-5047

Mayzaud P, Eaton CA, Ackman RG (1976) The occurence and distribution of octadecapentaenoic acid in a natural plankton population. A possible food chain index. Lipids 11:858-862

McCann K, Hastings A, Huxel GR (1998) Weak trophic interactions and the balance of nature. Nature 395:794-798

Meziane T, Bodineau L, Retiere C, Thoumelin G (1997) The use of lipid markers to define sources of organic matter in sediment and food web of the intertidal salt-marsh-flat ecosystem of Mont-Saint-Michel Bay, France. J Sea Res 38:47-58

Meziane T, Tsuchiya M (2000) Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. Mar Ecol Prog Ser 200:49-57

Middleboe M, Nielson TG, Bjørnsen PK (2002) Viral and bacterial production in the North Water: *in situ* measurements, batch-culture experiments and characterization and distribution of a virus-host system. Deep-Sea Res II 49:5063-5079

Nejstgaard JC, Hygum BH, Naustvoll L-J, Båmstedt U (2001) Zooplankton growth, diet and reproductive success compared in simultaneous diatom- and flagellate-microzooplankton dominated plankton blooms. Mar Ecol Prog Ser 221:77-91

Nelson MM, Mooney BD, Nichols PD, Phleger CF (2001) Lipids of Antarctic Ocean amphipods: food chain interactions and the occurence of novel biomarkers. Mar Chem 73:53-64

Norrbin MF, Olsen R-E, Tande KS (1990) Seasonal variation in lipid class and fatty acid composition of two small copepods in Balsfjorden, northern Norway. Mar Biol 105:205-211

Ohman MD, Runge JA (1994) Sustained fecundity when phytoplankton resources are in short supply: Omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. Limnol Oceanogr 39:21-36

Ohman MD (1997) On the determination of zooplankton lipid content and the occurrence of gelatinous copepods. J Plankton Res 19:1235-1250

Parrish CC (1987) Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can J Fish Aquat Sci 44:722-731

Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC (eds) Lipids in Freshwater Ecosystems. Springer-Verlag, New York, pp 4-20

Parrish CC, Abrajano TA, Budge SM, Helleur RJ, Hudson ED, Pulchan K, Ramos C (2000) Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky P (ed) The Handbook of Environmental Chemistry, vol. 5. Springer-Verlag, Berlin, pp 193-223

Pranal V, Fiala-Médioni A, Guezennec J (1996) Fatty acid characteristics in two symbiotic gastropods from a deep hydrothermal vent of the West Pacific. Mar Ecol Prog Ser 142:175-184

Ringuette M, Fortier L, Fortier M, Runge JA, Bélanger S, Larouche P, Weslawski J-M, Kwasniewski S (2002) Advanced recruitment and accelerated population development in Arctic calanoid copepods of the North Water. Deep-Sea Research II 49:5081-5099

Romano P, Feletti M, Mariottini GL, Carli A (1999) Ecological and nutritional implications of the mandibular structure in the Antarctic calanoid copepod *Metridia gerlachei* Giesbrecht, 1902: an ultrastructural study. Polar Biol 22:7-12

Sargent JR, Lee RF, Nevenzel JC (1976) Marine Waxes. In: Kolattukudy PE (ed) Chemistry and Biochemistry of Natural Waxes. Elsevier, Amsterdam, pp 49-91

Sargent JR, Eilersten HC, Falk-Petersen S, Taasen JP (1985) Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. Mar Biol 85:109-116

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleig MA (ed) Microbes in the Sea. Ellis Harwood, Chichester, pp 119-138

Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR (2002) Species differences, origins and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters. Mar Ecol Prog Ser 235:127-134

Stevens CJ, Deibel D, Parrish CC. Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with *Calanus glacialis* Jaschnov. J Exp Mar Biol Ecol (in press).

Viso A-C, Marty J-C (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34:1521-1533

White JR, Roman MR (1992) Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. Mar Ecol Prog Ser 86:239-249

Zhukova NV, Kharlamenko VI, Svetashev VI, Rodionov IA (1992) Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. J Exp Mar Biol Ecol 162:253-263

## 4.9 Tables

Table 4.1. Descriptions of North Water stations where samples of *Calanus hyperboreus* CV (C.h.), *C. glacialis* CV (C.g.) and *Metridia longa* females (M.l.) were collected in autumn 1999.

| Region[a] | Station # | Date (1999) | Depth (m) | Temperature Range[b] (°C) | Lipid Samples |
|---|---|---|---|---|---|
| Smith Sound (SS) | 02 | 11 Sept | 250-50 | -1.6 to -1.3 (s)<br>-1.5 to -0.6 (d) | C.h. (6,5,5)[c]<br>C.g. (5,5,10)<br>M.l. (22) |
| | 03 | 04 Sept | 250-60 | -1.3 to -0.4 (s)<br>-1.3 to -0.5 (d) | C.h. (4)<br>C.g. (4)<br>M.l. (25,24,24) |
| | 06 | 13 Sept | 150-75 | -1.1 to -0.7 (s)<br>-1.1 to -0.8 (d) | C.h. (6)<br>C.g. (10)<br>M.l. (15) |
| | 14 | 25 Sept | 300-100 | -1.4 to -0.7 (s)<br>-1.1 to -0.4 (d) | C.h. (5)<br>C.g. (9)<br>M.l. (24) |
| Central Northern Baffin Bay (CNBB) | 32 | 22 Sept | 195-75 | -1.5 to -0.7 (s)<br>-1.0 to -0.7 (d) | C.h. (5)<br>C.g. (9,10,10)<br>M.l. (17) |
| | 35 | 01 Sept | 250-60 | -1.3 to +1.5 (s)<br>-1.3 to +0.2 (d) | C.h. (4)<br>C.g. (8)<br>M.l. (20) |
| | 45 | 17 Sept | 250-75 | -1.5 to -0.4 (s)<br>-1.4 to -0.6 (d) | C.h. (3)<br>C.g. (9)<br>M.l. (20) |
| Carey Islands (CI) | 54 | 07 Sept | 250-75 | -0.8 to +2.3 (s)<br>-0.8 to 0.0 (d) | C.h. (4)<br>C.g. (7)<br>M.l. (25,23,20) |
| | 54 | 19 Sept | 250-75 | -0.9 to +0.7 (s)<br>-0.9 to +1.5 (d) | C.h. (2)<br>C.g. (10)<br>M.l. (23) |
| Station 58 | 58 | 08 Sept | 150-50 | -1.0 to +1.7 (s)<br>-1.0 to +0.1 (d) | C.h. (4)<br>C.g. (8)<br>M.l. (27) |
| West Greenland Current (WGC) | 66 | 29 Sept | 250-75 | -1.0 to +0.6 (s)<br>-1.0 to +1.0 (d) | C.h. (4)<br>C.g. (9)<br>M.l. (5) |
| | 76 | 01 Oct | 250-75 | -1.0 to +0.8 (s)<br>+0.1 to +2.4 (d) | C.h. (4,4,4)<br>C.g. (9)<br>M.l. (18) |

[a]after Lovejoy et al. (2002)
[b]approximate temperature range in surface (s) and deep (d) strata according to CTD traces; deep strata as in preceding column; surface strata from top of deep strata to surface
[c]numbers in brackets refer to copepods per sample

Table 4.2. Dry mass, lipid content and relative lipid class composition of *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* females in four regions of the North Water (abbreviations as in Table 4.1) and at Station 58 (TL=total lipid, HC=hydrocarbon, WE=wax ester, TG=triacylglycerol, AMPL=acetone-mobile polar lipid, PL=phospholipid; error estimates are 1 standard deviation).

| | Dry mass (mg ind$^{-1}$) | TL (mg ind$^{-1}$) | HC (%) | WE (%) | TG (%) | AMPL (%) | PL (%) | Neutral (%) | Polar (%) | % Lipid (wt) |
|---|---|---|---|---|---|---|---|---|---|---|
| **SS** | | | | | | | | | | |
| C.h. | 3.05 ± 0.93 | 1.53 ± 0.29 | 2.8 ± 0.3 | 84.2 ± 2.8 | 5.5 ± 2.9 | 2.3 ± 2.1 | 4.5 ± 1.3 | 93 | 7 | 50 |
| C.g. | 1.12 ± 0.36 | 0.57 ± 0.13 | 1.8 ± 0.4 | 82.8 ± 3.4 | 8.3 ± 3.2 | 2.0 ± 1.0 | 3.4 ± 0.8 | 95 | 5 | 51 |
| M.l. | 0.46 ± 0.05 | 0.16 ± 0.03 | 0.6 ± 0.3 | 71.9 ± 3.2 | 12.2 ± 2.1 | 3.4 ± 2.0 | 10.0 ± 1.3 | 87 | 13 | 35 |
| **CNBB** | | | | | | | | | | |
| C.h. | 2.93 ± 0.59 | 1.79 ± 0.29 | 1.7 ± 0.4 | 89.9 ± 2.7 | 2.9 ± 1.7 | 2.2 ± 1.1 | 2.5 ± 1.1 | 95 | 5 | 61 |
| C.g. | 1.39 ± 0.21 | 0.44 ± 0.07 | 1.9 ± 0.1 | 82.1 ± 4.0 | 9.1 ± 4.3 | 2.8 ± 1.3 | 2.5 ± 1.0 | 95 | 5 | 32 |
| M.l. | 0.52 ± 0.02 | 0.17 ± 0.04 | 0.4 ± 0.5 | 75.4 ± 3.5 | 12.7 ± 2.3 | 3.9 ± 0.4 | 6.4 ± 4.3 | 90 | 10 | 33 |
| **CI** | | | | | | | | | | |
| C.h. | 2.38 ± 1.18 | 1.67 ± 0.28 | 2.7 ± 1.4 | 89.5 ± 2.1 | 3.2 ± 0.0 | 1.3 ± 0.2 | 2.6 ± 0.4 | 96 | 4 | 70 |
| C.g. | 1.19 ± 0.19 | 0.60 ± 0.05 | 1.4 ± 0.3 | 88.3 ± 0.6 | 6.4 ± 1.7 | 1.5 ± 0.9 | 1.6 ± 0.6 | 97 | 3 | 51 |
| M.l. | 0.45 ± 0.05 | 0.06 ± 0.01 | 0.5 ± 0.0 | 74.4 ± 5.3 | 8.1 ± 4.6 | 3.1 ± 0.8 | 13.4 ± 3.7 | 83 | 17 | 14 |
| **STN 58** | | | | | | | | | | |
| C.h. | 3.23 | 1.48 | 1.4 | 93.1 | 0.4 | 2.2 | 2.5 | 95 | 5 | 46 |
| C.g. | 1.04 ± 0.18 | 0.55 | 1.4 | 90.0 | 5.6 | 0.8 | 1.9 | 97 | 3 | 53 |
| M.l. | 0.37 ± 0.03 | 0.04 | 0.2 | 49.3 | 4.6 | 5.0 | 37.7 | 57 | 43 | 11 |
| **WGC** | | | | | | | | | | |
| C.h. | 3.57 ± 0.40 | 1.76 ± 0.31 | 1.6 ± 0.6 | 90.2 ± 3.1 | 1.3 ± 1.2 | 1.7 ± 0.4 | 3.9 ± 0.7 | 94 | 6 | 49 |
| C.g. | 1.19 ± 0.06 | 0.69 ± 0.06 | 1.2 ± 0.4 | 85.9 ± 8.2 | 4.9 ± 3.6 | 2.1 ± 0.6 | 3.4 ± 1.5 | 95 | 5 | 58 |
| M.l. | 0.45 ± 0.01 | 0.14 ± 0.03 | 0.5 ± 0.3 | 73.4 ± 2.7 | 4.9 ± 1.8 | 3.0 ± 1.2 | 16.0 ± 0.3 | 81 | 19 | 30 |

Table 4.3. Relative fatty acid composition of *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* females in four regions of the North Water (abbreviations as in Table 4.1) and at Station 58 (OBFA=odd and/or branched fatty acids, SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids; error estimates are 1 standard deviation).

| Fatty acid[a] | | SS | | | CNBB | | | CI | |
|---|---|---|---|---|---|---|---|---|---|
| (%) | C.h. | C.g. | M.l. | C.h. | C.g. | M.l. | C.h. | C.g | M.l. |
| 14:0 | 2.8 ± 0.2 | 8.6 ± 0.7 | 2.0 ± 0.3 | 3.2 ± 0.5 | 8.3 ± 0.3 | 2.3 ± 0.5 | 3.1 ± 0.1 | 8.6 ± 0.2 | 1.7 ± 0.3 |
| i-15:0 | 0.1 ± 0.0 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.1 |
| ai-15:0 | tr | 0.1 ± 0.0 | 0.1 ± 0.0 | tr | 0.1 ± 0.0 | tr | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| 15:0 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.0 |
| 15:1 | 0.1 ± 0.0 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.0 | 0.2 ± 0.1 | 0.1 ± 0.0 | 0.3 ± 0.0 |
| 16:0 | 2.8 ± 0.3 | 5.9 ± 0.4 | 5.0 ± 0.4 | 2.9 ± 0.4 | 5.8 ± 0.3 | 5.8 ± 0.5 | 3.1 ± 0.7 | 6.1 ± 0.2 | 5.6 ± 0.7 |
| 16:1(n-7) | 20.4 ± 2.4 | 18.8 ± 2.4 | 28.7 ± 4.4 | 25.3 ± 4.1 | 20.3 ± 1.0 | 29.5 ± 6.7 | 21.8 ± 3.3 | 19.3 ± 1.0 | 24.9 ± 5.9 |
| i-17:0 | tr | 0.1 ± 0.1 | 0.1 ± 0.0 | tr | 0.1 ± 0.0 | 0.1 ± 0.1 | tr | 0.1 ± 0.0 | 0.2 ± 0.0 |
| ai-17:0 | 0.3 ± 0.0 | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.1 | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.3 ± 0.0 | 0.2 ± 0.0 |
| 16:2(n-4) | 1.5 ± 0.1 | 1.0 ± 0.1 | 0.5 ± 0.1 | 1.5 ± 0.1 | 1.0 ± 0.1 | 0.6 ± 0.1 | 1.6 ± 0.3 | 1.0 ± 0.0 | 0.5 ± 0.1 |
| 16:3(n-4) | 1.6 ± 0.2 | 1.6 ± 0.2 | 0.8 ± 0.1 | 1.4 ± 0.1 | 1.3 ± 0.1 | 0.6 ± 0.1 | 1.8 ± 0.4 | 1.5 ± 0.0 | 0.6 ± 0.0 |
| 17:1 | 0.1 ± 0.1 | tr | nd | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.2 | 0.1 ± 0.0 | 0.1 ± 0.0 | nd |
| 16:4(n-1) | 5.3 ± 0.6 | 5.2 ± 0.4 | 0.9 ± 0.1 | 4.1 ± 1.3 | 4.5 ± 0.4 | 1.0 ± 0.1 | 4.6 ± 1.6 | 4.8 ± 0.8 | 0.8 ± 0.3 |
| 18:0 | 0.2 ± 0.2 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.1 ± 0.0 | 0.2 ± 0.0 | 0.4 ± 0.1 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.4 ± 0.0 |
| 18:1(n-9) | 1.9 ± 0.3 | 2.5 ± 0.4 | 12.3 ± 1.1 | 2.1 ± 0.6 | 1.9 ± 0.4 | 13.2 ± 1.4 | 1.8 ± 0.0 | 2.5 ± 0.3 | 13.3 ± 2.3 |
| 18:1(n-7) | 1.2 ± 0.1 | 0.8 ± 0.1 | 2.2 ± 0.4 | 1.4 ± 0.2 | 0.8 ± 0.1 | 2.2 ± 0.2 | 1.2 ± 0.1 | 0.9 ± 0.0 | 1.9 ± 0.5 |
| 18:1(n-5) | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.0 | 0.5 ± 0.0 | 0.3 ± 0.0 | 0.4 ± 0.1 | 0.5 ± 0.2 | 0.4 ± 0.0 | 0.7 ± 0.4 |
| 18:2(n-6) | 0.6 ± 0.1 | 0.4 ± 0.1 | 1.2 ± 0.1 | 0.9 ± 0.3 | 0.5 ± 0.2 | 1.2 ± 0.1 | 1.0 ± 0.1 | 0.6 ± 0.1 | 1.4 ± 0.3 |
| 18:3(n-3) | 0.2 ± 0.0 | 0.3 ± 0.1 | 0.6 ± 0.1 | 0.3 ± 0.1 | 0.2 ± 0.0 | 0.4 ± 0.0 | 0.3 ± 0.0 | 0.3 ± 0.1 | 0.4 ± 0.1 |
| 18:4(n-3) | 1.8 ± 0.2 | 2.2 ± 0.2 | 2.5 ± 0.2 | 2.7 ± 1.8 | 1.9 ± 0.2 | 2.5 ± 0.4 | 3.1 ± 1.0 | 2.7 ± 0.6 | 2.7 ± 0.2 |
| 20:1(n-9) | 7.0 ± 1.1 | 9.7 ± 0.9 | 3.7 ± 0.8 | 5.8 ± 0.8 | 7.0 ± 1.7 | 3.7 ± 1.6 | 5.9 ± 2.4 | 9.3 ± 2.0 | 4.3 ± 3.9 |
| 20:1(n-7) | 1.1 ± 0.3 | 0.2 ± 0.0 | 0.3 ± 0.1 | 1.4 ± 0.4 | 0.2 ± 0.1 | 0.4 ± 0.5 | 1.0 ± 0.2 | 0.2 ± 0.0 | 1.6 ± 2.8 |
| 20:4(n-6) | 0.6 ± 0.1 | 0.4 ± 0.1 | 0.6 ± 0.2 | 0.6 ± 0.1 | 0.5 ± 0.0 | 1.3 ± 1.2 | 0.5 ± 0.0 | 0.5 ± 0.0 | 0.7 ± 0.4 |
| 20:4(n-3) | 0.9 ± 1.1 | 0.3 ± 0.0 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.0 | 0.6 ± 0.3 | 1.2 ± 1.3 | 0.4 ± 0.0 | 3.3 ± 4.9 |
| 20:5(n-3) | 28.6 ± 4.0 | 24.5 ± 2.2 | 17.3 ± 0.9 | 25.5 ± 5.5 | 30.4 ± 2.5 | 16.1 ± 2.2 | 28.1 ± 2.7 | 25.9 ± 3.8 | 16.3 ± 2.9 |
| 22:1(n-11) | 6.5 ± 1.0 | 5.9 ± 1.5 | 1.5 ± 0.5 | 6.3 ± 1.2 | 4.6 ± 0.6 | 2.2 ± 0.0 | 5.0 ± 1.8 | 4.8 ± 1.0 | 2.1 ± 0.5 |
| 22:1(n-9) | 1.8 ± 0.4 | 0.8 ± 0.3 | 0.3 ± 0.1 | 1.4 ± 0.4 | 0.7 ± 0.3 | 0.5 ± 0.2 | 1.0 ± 0.8 | 0.6 ± 0.1 | 0.5 ± 0.0 |
| 21:5(n-3) | 0.1 ± 0.2 | 0.7 ± 0.4 | 0.9 ± 0.7 | 0.1 ± 0.2 | 0.3 ± 0.0 | 0.3 ± 0.2 | 1.0 ± 1.2 | 0.4 ± 0.0 | 0.4 ± 0.4 |
| 22:5(n-3) | 1.8 ± 0.1 | 1.0 ± 0.4 | 1.4 ± 1.1 | 1.9 ± 0.6 | 1.0 ± 0.1 | 0.9 ± 0.8 | 1.9 ± 0.2 | 0.9 ± 0.2 | 0.7 ± 0.2 |
| 22:6(n-3) | 5.7 ± 1.6 | 4.0 ± 1.9 | 9.9 ± 5.0 | 5.3 ± 2.2 | 3.7 ± 1.0 | 7.1 ± 5.5 | 4.8 ± 1.4 | 3.7 ± 1.6 | 8.0 ± 0.2 |
| 24:1 | 0.5 ± 0.3 | 0.6 ± 0.6 | 2.0 ± 1.2 | 0.4 ± 0.2 | 0.3 ± 0.1 | 1.2 ± 0.6 | 0.6 ± 0.4 | 0.3 ± 0.0 | 0.7 ± 0.1 |
| OBFA | 0.8 ± 0.1 | 1.2 ± 0.3 | 0.9 ± 0.2 | 0.7 ± 0.1 | 1.3 ± 0.4 | 1.2 ± 0.2 | 0.8 ± 0.4 | 1.4 ± 0.3 | 1.2 ± 0.2 |
| SFA | 6.6 ± 0.3 | 15.7 ± 1.4 | 8.0 ± 0.6 | 7.1 ± 1.1 | 15.4 ± 0.5 | 9.5 ± 1.0 | 7.5 ± 0.9 | 16.2 ± 0.8 | 9.0 ± 1.3 |
| MUFA | 41.8 ± 3.7 | 40.7 ± 2.0 | 52.2 ± 5.2 | 45.7 ± 6.1 | 37.2 ± 2.8 | 54.3 ± 8.0 | 39.9 ± 1.9 | 39.2 ± 4.7 | 51.0 ± 2.5 |
| PUFA | 51.6 ± 3.9 | 43.6 ± 3.1 | 39.8 ± 5.6 | 47.3 ± 6.9 | 47.4 ± 2.9 | 36.2 ± 8.5 | 52.6 ± 1.0 | 44.6 ± 5.5 | 39.9 ± 3.6 |
| (n-3) | 39.4 ± 3.9 | 33.4 ± 3.4 | 33.1 ± 6.0 | 36.6 ± 6.0 | 38.3 ± 2.8 | 27.7 ± 8.5 | 40.7 ± 2.4 | 34.7 ± 4.8 | 32.2 ± 3.3 |
| (n-6) | 1.8 ± 0.3 | 1.4 ± 0.2 | 2.7 ± 0.6 | 2.0 ± 0.7 | 1.5 ± 0.2 | 4.3 ± 2.2 | 1.9 ± 0.0 | 1.5 ± 0.2 | 3.0 ± 0.2 |

[a]data not shown for fatty acids (excluding odd and/or branched chains) <1% total in all samples (i.e., 14:1, 16:1(n-5), 16:4(n-3), 18:2(n-4), 18:3(n-6), 18:3(n-4), 18:4(n-1), 20:0, 20:2(n-6), 20:3(n-6), 20:3(n-3), 22:1(n-7); see Appendices 7-12); tr=trace (<0.1% total); nd=not detected

Table 4.3. Continued.

| Fatty acid | STN 58 | | | WGC | | |
|---|---|---|---|---|---|---|
| (%) | C.h. | C.g. | M.l. | C.h. | C.g. | M.l. |
| 14:0 | 4.6 | 8.4 | 2.7 | 3.6 ± 0.3 | 9.2 ± 0.7 | 1.4 ± 0.5 |
| i-15:0 | 0.2 | 0.2 | 0.2 | 0.1 ± 0.0 | 0.3 ± 0.0 | nd |
| ai-15:0 | 0.3 | 0.1 | 0.4 | 0.1 ± 0.1 | 0.1 ± 0.0 | nd |
| 15:0 | 0.4 | 0.3 | 1.1 | 0.2 ± 0.1 | 0.3 ± 0.1 | 0.1 ± 0.1 |
| 15:1 | 0.1 | 0.1 | 1.0 | 0.1 ± 0.1 | 0.2 ± 0.0 | tr |
| 16:0 | 5.3 | 6.6 | 13.1 | 3.8 ± 0.5 | 6.8 ± 1.0 | 5.6 ± 1.4 |
| 16:1(n-7) | 22.3 | 23.8 | 19.1 | 19.3 ± 6.2 | 21.2 ± 3.6 | 15.0 ± 1.4 |
| i-17:0 | 0.1 | 0.1 | 0.1 | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.2 ± 0.2 |
| ai-17:0 | 0.2 | 0.3 | nd | 0.2 ± 0.0 | 0.2 ± 0.0 | 0.2 ± 0.3 |
| 16:2(n-4) | 1.3 | 0.7 | 0.6 | 1.0 ± 0.3 | 0.9 ± 0.2 | 0.8 ± 0.6 |
| 16:3(n-4) | 0.7 | 1.0 | 0.8 | 0.9 ± 0.4 | 1.2 ± 0.4 | 0.2 ± 0.1 |
| 17:1 | 0.1 | nd | nd | nd | 0.1 ± 0.1 | nd |
| 16:4(n-1) | 1.6 | 1.7 | 0.3 | 1.5 ± 0.9 | 2.1 ± 1.1 | 0.6 ± 0.4 |
| 18:0 | 0.4 | 0.3 | 1.1 | 0.3 ± 0.1 | 0.4 ± 0.1 | 1.0 ± 0.4 |
| 18:1(n-9) | 3.9 | 4.6 | 12.7 | 2.9 ± 0.7 | 3.7 ± 1.2 | 13.6 ± 4.1 |
| 18:1(n-7) | 1.8 | 0.8 | 3.2 | 1.4 ± 0.0 | 0.8 ± 0.1 | 1.6 ± 0.2 |
| 18:1(n-5) | 1.0 | 0.4 | 1.3 | 0.6 ± 0.1 | 0.4 ± 0.1 | 0.9 ± 0.3 |
| 18:2(n-6) | 3.5 | 1.2 | 2.2 | 2.0 ± 0.8 | 0.9 ± 0.5 | 3.6 ± 1.9 |
| 18:3(n-3) | 1.6 | 0.8 | 0.5 | 1.3 ± 0.7 | 0.7 ± 0.2 | 0.9 ± 0.1 |
| 18:4(n-3) | 10.1 | 5.7 | 1.6 | 8.7 ± 4.1 | 3.6 ± 1.1 | 3.7 ± 0.9 |
| 20:1(n-9) | 3.1 | 12.0 | 7.2 | 10.5 ± 2.5 | 9.3 ± 1.0 | 3.5 ± 1.6 |
| 20:1(n-7) | 0.2 | 0.2 | 0.2 | 1.3 ± 0.3 | 0.3 ± 0.0 | 0.5 ± 0.7 |
| 20:4(n-6) | 0.3 | 0.3 | 0.2 | 0.4 ± 0.0 | 0.4 ± 0.0 | 0.3 ± 0.1 |
| 20:4(n-3) | 1.1 | 0.7 | nd | 0.7 ± 0.6 | 0.6 ± 0.2 | 0.5 ± 0.1 |
| 20:5(n-3) | 10.9 | 14.1 | 11.1 | 12.7 ± 3.3 | 15.7 ± 6.6 | 10.9 ± 4.0 |
| 22:1(n-11) | 8.0 | 5.3 | 2.9 | 7.3 ± 1.3 | 5.9 ± 0.8 | 1.1 ± 0.5 |
| 22:1(n-9) | 1.9 | 0.9 | 0.4 | 2.4 ± 0.6 | 0.6 ± 0.0 | 0.1 ± 0.2 |
| 21:5(n-3) | 0.2 | 0.3 | nd | 0.3 ± 0.1 | 1.4 ± 0.9 | 0.5 ± 0.7 |
| 22:5(n-3) | 1.1 | 0.6 | 0.2 | 1.6 ± 0.8 | 1.2 ± 0.3 | 3.0 ± 2.0 |
| 22:6(n-3) | 7.9 | 4.9 | 9.8 | 9.8 ± 1.2 | 6.9 ± 1.0 | 19.1 ± 4.3 |
| 24:1 | 0.4 | 0.4 | 1.9 | 0.8 ± 0.5 | 1.8 ± 0.2 | 6.4 ± 3.0 |
| | | | | | | |
| OBFA | 1.6 | 1.3 | 3.9 | 0.9 ± 0.3 | 1.4 ± 0.3 | 0.8 ± 0.4 |
| SFA | 12.2 | 16.5 | 20.2 | 8.5 ± 0.9 | 17.7 ± 1.7 | 9.5 ± 2.1 |
| MUFA | 44.5 | 49.5 | 50.8 | 47.9 ± 1.8 | 45.0 ± 5.5 | 44.6 ± 6.5 |
| PUFA | 43.3 | 33.9 | 29.0 | 43.5 ± 1.3 | 37.3 ± 7.2 | 45.9 ± 8.6 |
| (n-3) | 33.5 | 27.5 | 23.1 | 35.5 ± 2.1 | 30.3 ± 7.1 | 38.6 ± 8.4 |
| (n-6) | 4.6 | 2.3 | 2.7 | 3.3 ± 0.8 | 2.0 ± 0.9 | 4.8 ± 1.2 |

## 4.10 Figures

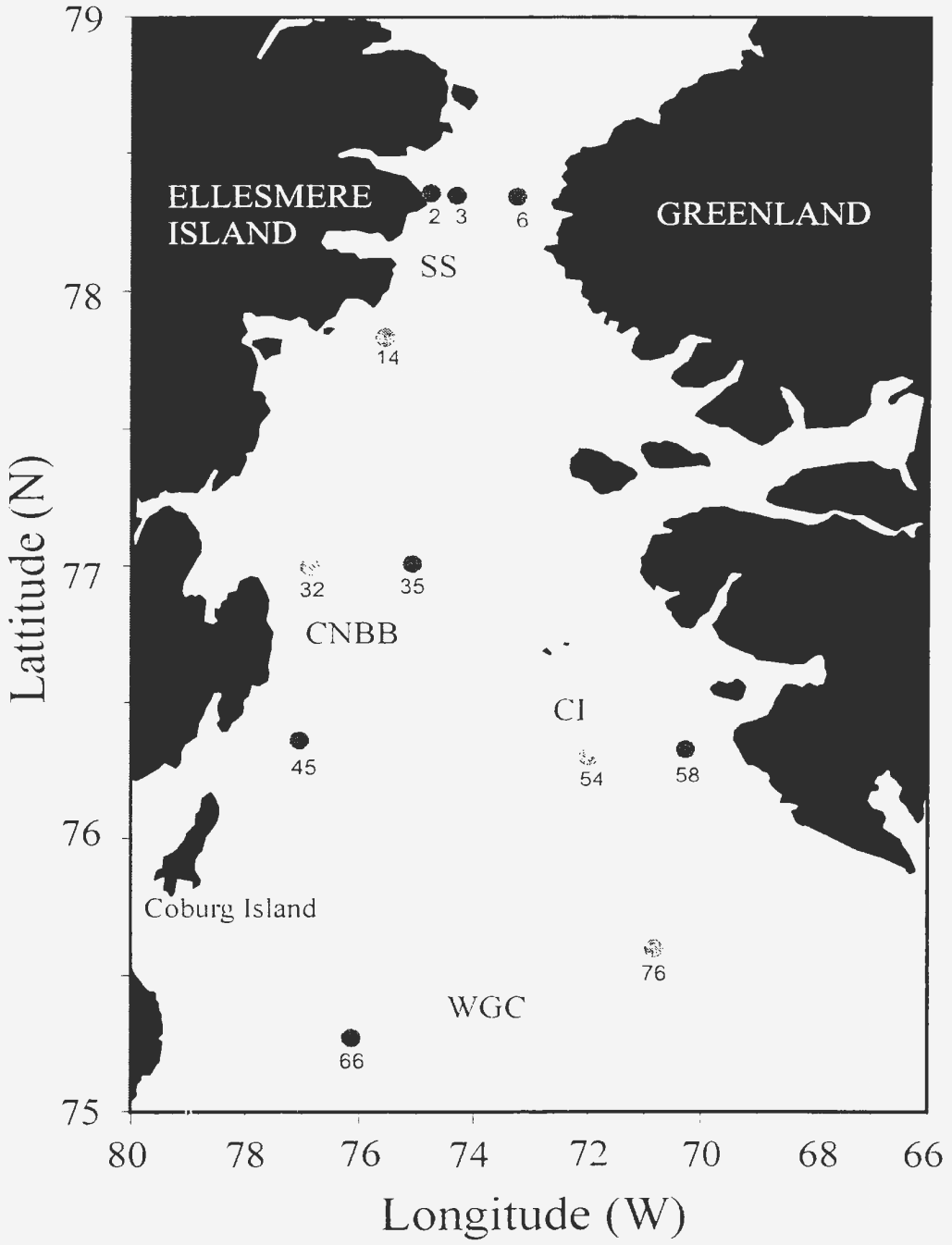



Figure 4.1. Stations sampled below the mixed layer in the North Water Polynya during autumn 1999 (Regional groupings after Lovejoy et al. (2002) where SS=Smith Sound, CNBB=Central Northern Baffin Bay, CI=Carey Islands and WGC=West Greenland Current).

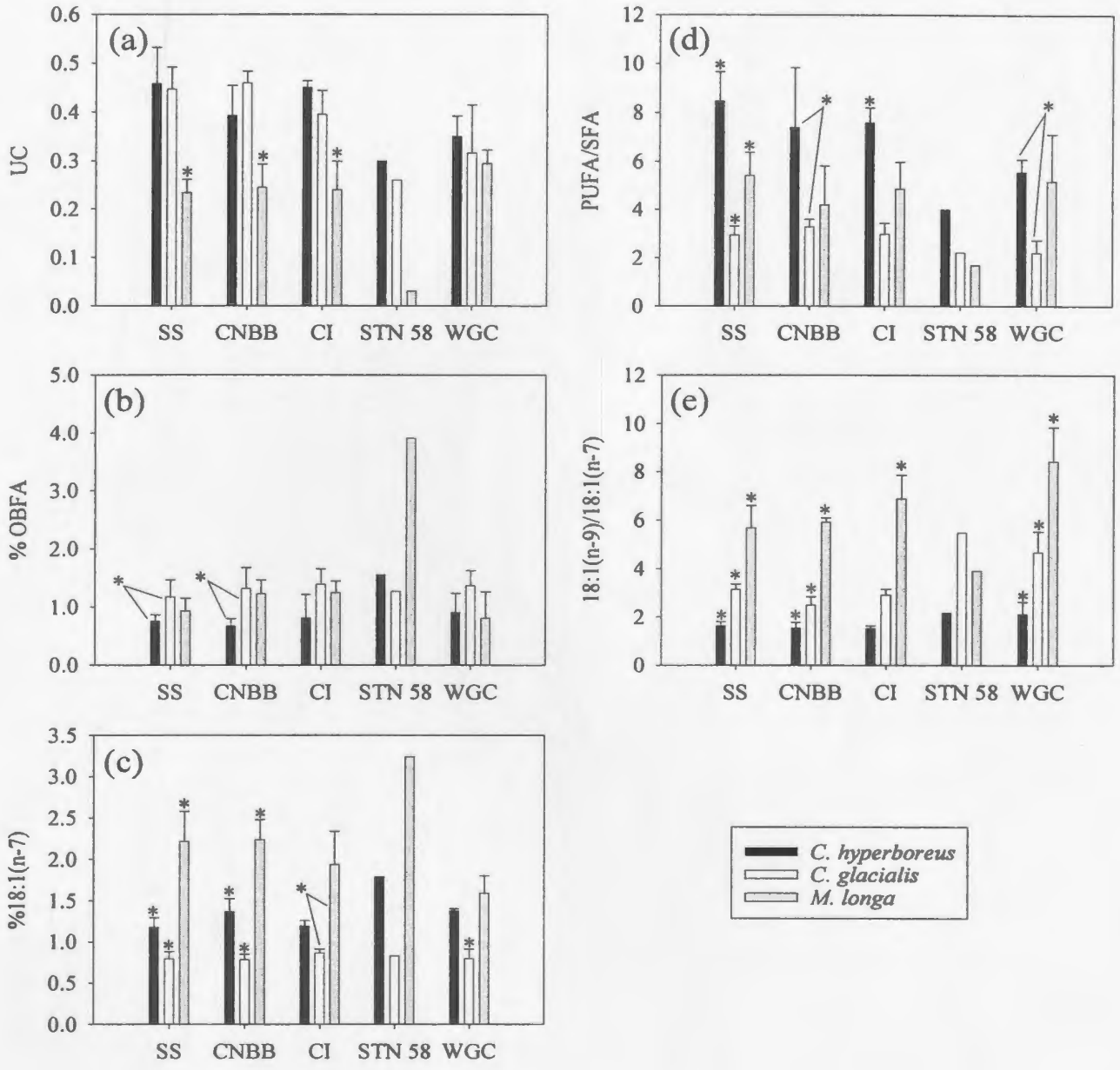



Figure 4.2. Values of several dietary indices in *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* females from four regions of the North Water (regional abbreviations as in Figure 4.1), and at Station 58, sampled in autumn 1999 (UC=unsaturation coefficient; PUFA=polyunsaturated fatty acids; SFA=saturated fatty acids; error bars represent 1 standard deviation; '*' denotes significance at $\alpha$=0.05).

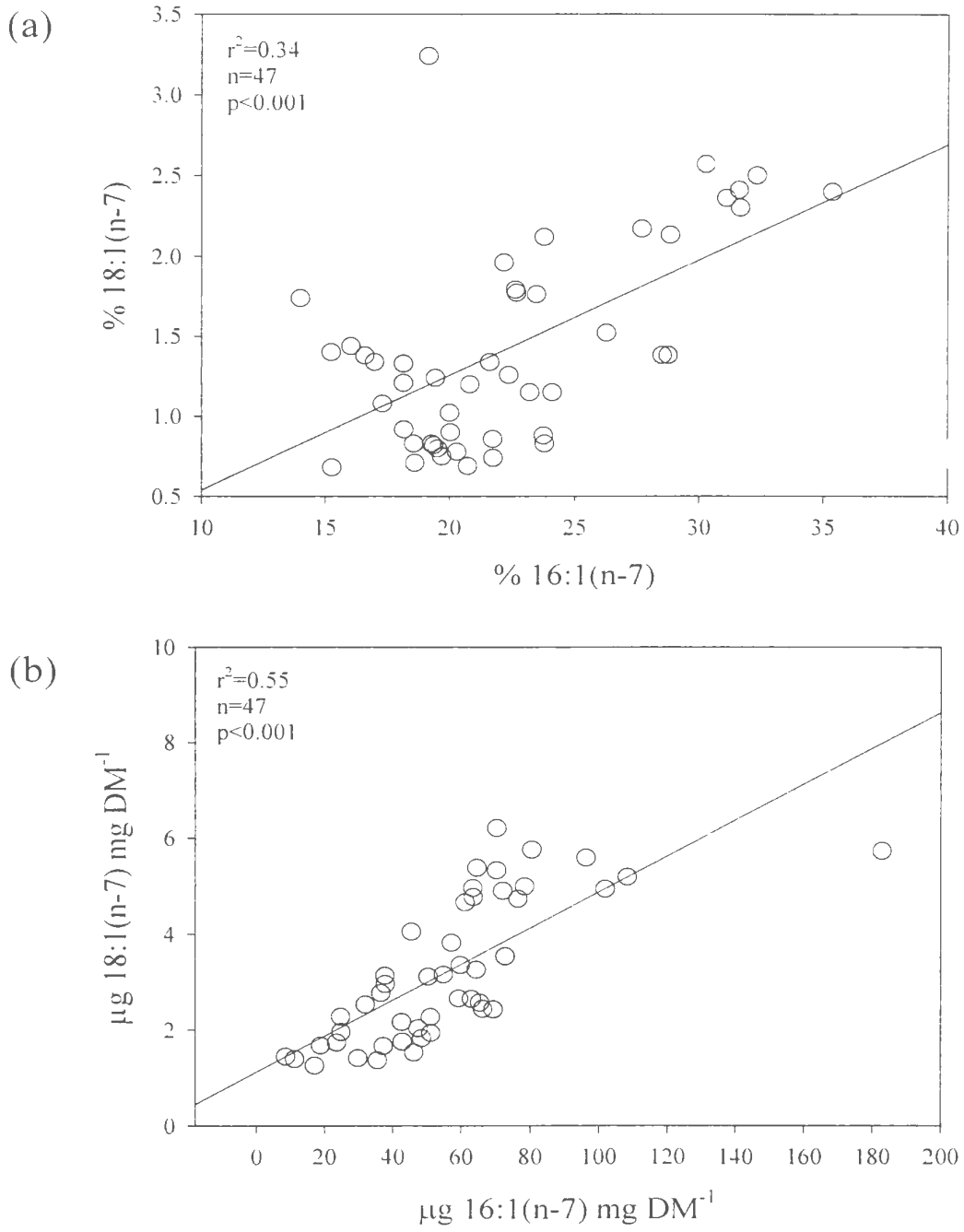
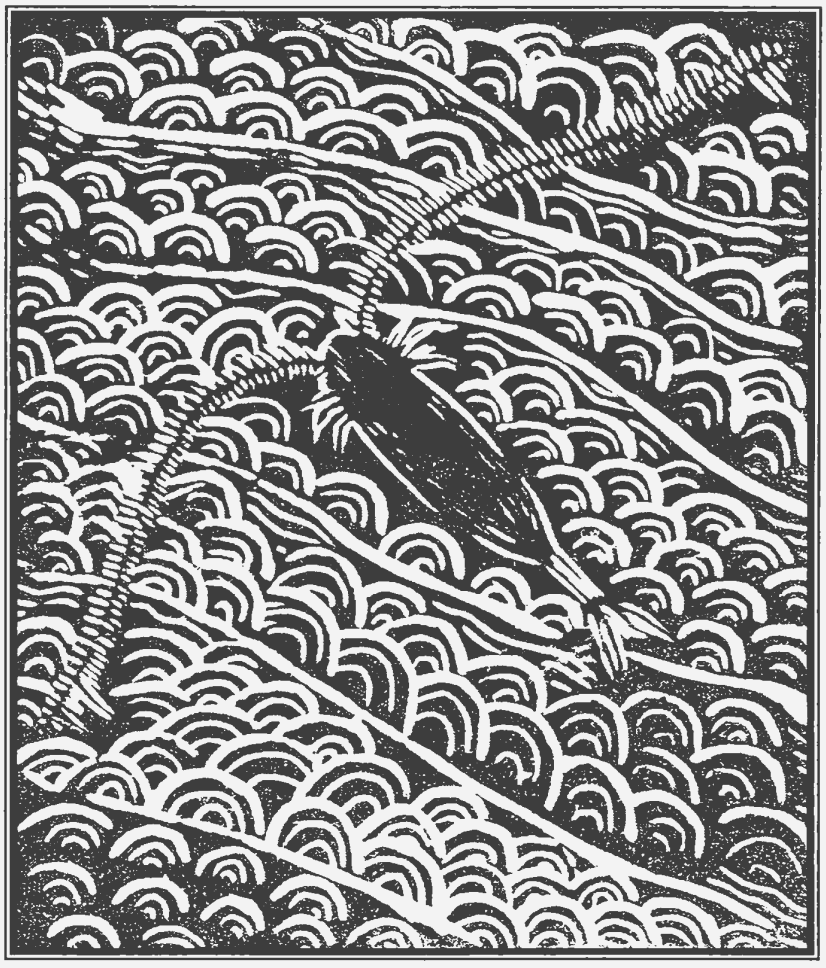



Figure 4.3. Relationship between (a) relative (%) and (b) absolute (µg mg $DM^{-1}$; DM=dry mass) amounts of 16:1(n-7) and 18:1(n-7) in copepods collected during autumn in the North Water (regression statistics in upper left hand corner of each panel).

# Chapter 5. Copepod omnivory in the North Water Polynya during autumn: spatial patterns in lipid composition



## 5.1 Abstract

To deduce spatial patterns in copepod lipid composition and feeding strategy in the North Water Polynya, three dominant species were sampled extensively over a broad geographical area (~75-78°N; 77-69°W). *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* females were collected in shallow and deep strata at 16 stations during autumn. Principal components analysis revealed that all species fed omnivorously in the southeastern (SE) region of the polynya. Here, copepods generally had elevated levels of carnivorous (e.g., 18:1(n-9)), dinoflagellate (e.g., 18:4(n-3); 22:6(n-3)) and bacterial fatty acid markers (e.g., odd-numbered and/or branched; 18:1(n-7)). Copepods in the SE contained low proportions of diatom (e.g., 16:4(n-1); 20:5(n-3)) and phytoplankton (e.g., polyunsaturated fatty acids) markers, relative to animals from northwest stations. Values of the omnivory index "UC" (unsaturation coefficient) were also low in the SE, which implied reduced phytoplankton ingestion. Spatial patterns in seston fatty acid composition resembled the dietary signatures in that dinoflagellate and bacterial indices were highest in the SE. Estimates of primary production, particulate organic carbon, carbon to chlorophyll ratios, and abundances of diatoms, dinoflagellates and bacteria, provided further evidence of the importance of the microbial loop at SE stations. Similar spatial patterns in feeding strategy were observed in both sampling layers, indicating that copepods from the entire water column were feeding on a similar food source.

## 5.2 Introduction

Fatty acids have been used extensively in zooplankton ecological studies to determine trophic relationships and diet (e.g., Falk-Petersen et al. 1987; Kattner et al. 1989; Desvilettes et al. 1994; Cripps and Hill 1998; Scott et al. 1999; Falk-Petersen et al. 2000; Nelson et al. 2001; Auel et al. 2002). These compounds are suitable dietary biomarkers because they can differentiate between prey groups (Ackman and Tocher 1968; Volkman et al. 1989; Viso and Marty 1993), they are conservative (Lee et al. 1971; Weers et al. 1997) and are essential for optimal growth and development (Brett and Müller-Navarra 1997; DeMott and Müller-Navarra 1997).

The fatty acids 16:1(n-7), 20:5(n-3) (eicosapentaenoic acid; EPA), 16:4(n-1), 16:2(n-7) and 16:2(n-4) are diatom biomarkers (Viso and Marty 1993). Principal dinoflagellate indicators are 18:4(n-3) and 22:6(n-3) (docosahexaenoic acid; DHA) (Viso and Marty 1993). Diatoms thus contain primarily $C_{16}$ and $C_{20}$ PUFA (polyunsaturated fatty acids), while dinoflagellates are characterized by $C_{18}$ and $C_{22}$ PUFA. DHA to EPA ratios have been used to show dominance of dinoflagellates over diatoms in the seston (Budge and Parrish 1998) and may also be applicable to zooplankton. Odd and/or branched fatty acids (OBFA) and (n-7) and (n-9) monounsaturates (e.g., 18:1(n-7)) are considered markers of bacteria (Sargent et al. 1987; Kaneda 1991; Pranal et al. 1996 and references therein). The fatty acid 18:5(n-3) has been used as a marker of both prymnesiophytes (Sargent et al. 1985; Pond et al. 1998) and dinoflagellates (Mayzaud et

al. 1976). Relative proportions of the above acids in zooplankton tissues have been used to infer diet in many aquatic environments (e.g., Sargent et al. 1985; Kattner et al. 1989; Desvilettes et al. 1994; Nelson et al. 2001).

Broad feeding strategies of zooplankton can also be established using fatty acid composition. High ratios between (n-3) and (n-6) PUFA, as well as 16:1(n-7)/16:0 > 1, are considered indications of herbivory in copepods (Sargent and Falk-Petersen 1981; Graeve et al. 1994). In addition, copepods feeding herbivorously generally contain higher proportions of PUFA than those feeding omnivorously or carnivorously (Falk-Petersen et al. 1987; Graeve et al. 1994). Monounsaturated fatty acid distributions are also related to feeding strategy. Copepods feeding herbivorously contain high relative amounts of 20:1(n-9) and 22:1(n-11), and comparatively little 18:1(n-9) (Graeve et al. 1994). The fatty acids 20:1(n-9) and 22:1(n-11) are thought to be formed *de novo* by polar copepods via desaturation and elongation of saturated fatty acids originating from a phytoplankton-dominated diet (Kattner and Hagen 1995).

Fatty acid biomarkers are particularly useful in monitoring diet over large geographical areas where gradients in microplankton assemblages are often encountered. Copepods collected in open water *versus* marginal ice zones (MIZ) in the Arctic and Antarctic had distinct lipid compositions that closely mirrored the available prey (Kattner et al. 1989; Cripps and Hill 1998). In these studies, copepods at bloom stations contained

high proportions of diatom biomarkers and were lipid-rich. Conversely, animals from the MIZ, largely exposed to flagellates, contained high proportions of 18:4(n-3) and 22:6(n-3). It was also shown that omnivory was more common in low chlorophyll environments. These results imply that copepods eat the most abundant prey. In response to phytoplankton shortage, copepods often switch to alternate prey including nauplii (Landry 1981), ciliates (Atkinson 1996) and heterotrophic dinoflagellates (Levinsen et al. 2000).

In large-scale spatial studies where many biological and environmental variables are simultaneously measured, multivariate statistics can aid greatly with data interpretation and illustration of important differences among samples. Tools such as principal components analysis and cluster analysis have been used to determine sources of organic matter in estuarine environments (Yunker et al. 1995; Colombo et al. 1996), establish causal relationships between biological processes and environmental conditions (Jónasdóttir et al. 1995), delineate biotic communities (Field et al. 1982; Mees and Hamerlynk 1992) and identify zooplankton feeding strategies (Hopkins 1987; Hopkins and Torres 1989; Albers et al. 1996; Cripps and Hill 1998).

This chapter is part of the *International North Water Polynya Study*, a multi-disciplinary investigation into the workings of a remote polar system. The North Water is a large, productive, recurring polynya in northern Baffin Bay that supports large seabird

and marine mammal populations (Deming et al. 2002). Inflows from three principal points converge to produce the water column structure characteristic of the North Water: (1) Arctic inflow via Smith Sound in the north and (2) Jones Sound in the southwest, and (3) the West Greenland Current in the southeast (Bâcle et al. 2002). These water masses are associated with distinct microplankton communities (Lovejoy et al. 2002a) and varying levels of phytoplankton biomass (Klein et al. 2002) and diatom productivity (Tremblay et al. 2002). It is proposed that these prey gradients will produce spatially distinct groups of copepods with different lipid compositions and feeding strategies.

Although a number of primary trophic linkages have been established throughout the North Water (Acuña et al. 2002; Karnovsky and Hunt 2002; Hobson et al. 2002; Middleboe et al. 2002), copepod diet has not yet been rigorously investigated in a spatial context. This chapter reports the lipid compositions of three dominant arctic copepods (*Calanus hyperboreus* CV, *C. glacialis* CV, *Metridia longa* CVI (female)) collected at numerous stations in the North Water. Using principal components and cluster analyses, I classified regions of the polynya dominated by herbivory and omnivory and identified primary dietary items in each. These analyses also allowed assessment of spatial changes in bacterial dietary input, diapause and general copepod health.

## 5.3 Materials and Methods

### *5.3.1 Field sampling*

From 27 August to 01 October 1999, copepods were collected at 16 stations in the North Water polynya (Table 5.1; Figure 5.1). Animals were collected from one or two discrete strata using a messenger-activated, closing net system equipped with 200 μm mesh nets and partially closed codends. All nets were towed vertically and were retrieved at a speed of 0.3-0.5 m $s^{-1}$. CTD (conductivity, temperature, depth) and fluorescence profiles were used to determine sampling strata. Generally, upper strata (e.g., 75-0 m) were above the thermocline and contained peak fluorescence values while deep strata (e.g., 250-75 m) were below the thermocline and were characterized by low, uniform fluorescence values.

Single or triplicate samples of *Calanus hyperboreus* CV (2-8 individual $sample^{-1}$), *C. glacialis* CV (3-11 ind. $sample^{-1}$) and *Metridia longa* females (5-27 ind. $sample^{-1}$) were picked out of the catches using the blunt end of a pipette, a dissecting microscope, and a 10 ml sorting cell (Table 5.1). Sorted copepods were placed in specimen cups filled with ~50 ml filtered seawater (0.2 μm) and kept on ice. After a sufficient number was picked, the contents of the cups were filtered onto combusted 25 mm GF/C filters and then folded, quick-frozen on an aluminum block (pre-cooled to -80°C), placed in combusted foil envelopes, and stored at -80°C. Unsorted assemblages of copepods (~200-300 individuals) were also taken from each net tow, collected on combusted 47 mm GF/C

filters, quick-frozen, and stored as above. Later, filters were thawed and copepods were removed and sorted for dry mass analysis and isolation of wax ester (see sections 5.3.2 and 5.3.3).

Seston samples were collected using a rosette (General Oceanics Inc.) equipped with 10-l Niskin bottles and a CTD (ICTD, Falmouth Scientific Inc.). Water from depths corresponding to copepod sampling strata (i.e., the 1% light depth and within the deep stratum (e.g., 200 m)) was collected at most stations. The 1% light depth was determined aboard ship using a Secchi disk, *in situ* PAR (photosynthetically available radiation) profiles, or *a posteriori* using established relationships between the euphotic depth (1% light) and chlorophyll concentration in the euphotic zone (see Klein et al. 2002). Because different methods of determination were used, shallow seston sampling depths did not always correspond to the 1% light depths (compare columns 3 & 7 of Table 5.1). Water was gently drained from the bottles with silicon tubing and collected in 10-l polycarbonate carboys. Between 3.2 and 5.3 l seawater from each depth were collected on combusted 47 mm GF/F filters, quick-frozen on the aluminum block and stored at -80°C. Although rare, any visible zooplankton retained by the filters were removed and discarded.

*5.3.2 Dry mass analysis*

Between 1 and 5 *Calanus hyperboreus* CV, 1-8 *C. glacialis* CV and 1-13 *Metridia longa* females were gently removed from thawed filters and placed in pre-weighed, combusted tin cups. Where possible (> 80% of the time), 1-2 additional replicate samples were taken. The tin cups were then dried at ~60°C and weighed on a Mettler Toledo microbalance (UMT2).

*5.3.3 Wax ester isolation*

Forty-eight *Calanus hyperboreus* (females, CV, CIV, CIII) were removed from filters collected at Station 06, placed in 2 ml chloroform and total lipids extracted (see section 5.3.4). Following Ohman (1997), lipids were dried, re-suspended in hexane, and applied to a combusted glass column with a 6 ml bed volume of silica gel. Wax esters (WE) were eluted with 45 ml 1% diethyl ether in hexane, dried under a stream of $N_2$, and weighed to constant mass. This native WE (95% pure) was then used to calibrate the Iatroscan. Stevens et al. (in press 'a') found that during Iatroscan development, WE in *C. hyperboreus* split according to the degree of unsaturation of acyl lipid. The first peak contained predominantly saturated and monounsaturated fatty acids, whereas the second contained mostly polyunsaturated fatty acids. Unsaturation coefficients (UC; Stevens et al. in press 'a') were calculated as the ratio of the second WE peak area to that of total WE (peak 1 + peak 2). UC have been shown to reflect diet: high values in copepods

correspond to herbivory and low values to ingestion of microbial prey (Stevens et al. in press 'a').

*5.3.4 Determination of lipid classes and fatty acids*

Samples were brought to the laboratory on ice and placed in 2 ml chloroform. Lipids were extracted following Parrish (1999). To separate and quantify lipid classes, samples were manually spotted on silica-coated Chromarods (SIII), developed, and passed through the flame ionization detector (FID) of an Iatroscan MK V. The air and hydrogen flow rates were set to 2 l $min^{-1}$ and 190 ml $min^{-1}$, respectively. Rod development was done following Parrish (1987) where WE were resolved by double development in a non-polar solvent system (hexane:diethyl ether:formic acid (99:1:0.05), 25 min + 20 min). Commercial standards (Sigma-Aldrich Canada Ltd.) were used to calibrate the Iatroscan and establish peak identities.

Fatty acids were quantified as methyl esters by FID using a Varian 3400 gas chromatograph (GC), following total lipid derivatization of samples with $BF_3$-methanol (85°C, 1 h). Methyl esters were analyzed on an Omegawax column following Budge and Parrish (1998). Tricosanoic acid (23:0) was used as an internal standard at a concentration of ~10% total fatty acids. Peaks were identified by comparing sample retention times to those of commercial standard mixtures (Supelco, Sigma-Aldrich Canada Ltd.) following Ackman (1986), and by using a Varian 2000 GC/mass

spectrometer. The term "odd and/or branched fatty acids" (OBFA) is used to describe those fatty acids that have odd-numbered carbon chains (with the exception of 21:5(n-3)) and/or iso or anteiso branches.

*5.3.5 Statistical analysis*

Principal components analysis (PCA; SPSS 9.0) was used to elucidate spatial patterns in copepod lipid composition and lipid-based omnivory indices. Because *Calanus* spp. and *Metridia* spp. have unique lipid compositions related to feeding strategy (Stevens et al. in press 'b'), species were considered separately in order to maximize spatial resolution. Tables 5.2-5.6 show all of the variables that were significantly correlated with either of the first two principal components ($r \geq |0.7|$; Meglen 1992) or were of particular interest (i.e., omnivory indices UC, 18:1(n-7), ΣOBFA). Collectively, the variables in these tables account for most of those included in all PCA (see Appendix 15 for all variables included in each analysis and Appendices 1-12 for corresponding raw data). Following Meglen (1992), variables with missing values in > 30% of cases were excluded from analyses; otherwise, missing values were replaced with variable means and cases below detection limits were included as zeros. In the interest of space and clarity, estimates of variability associated with triplicate samples (see Table 5.1) were not shown in Tables 5.2-5.6 (overall, individual coefficients of variation (cv) ranged between 0.05 and 173.21; mean cv ranged between 21.90 and 46.74; see raw data in Appendices 1-12). Prior to analysis, all variables were standardized (z-scores).

PCA loadings are the correlation coefficients between the original variables and the *principal components* – linear, orthogonal combinations of these variables that summarize natural associations within the data (Meglen 1992). Loadings (e.g., Figures 5.2a - 5.6a) thus show relationships among *variables*. PCA scores are the positions of the original variables along the new axes (principal components; PC). Graphical representations of the scores on these axes (e.g., Figures 5.2b - 5.6b) show relationships among *samples* (Meglen 1992). The loadings matrix is used in the derivation of scores, thus variables with high loadings are the most influential in the calculation of scores. Discrete station groups (Figures 5.2b - 5.6b) were objectively delineated by performing hierarchical cluster analysis (CA; SPSS 9.0) on PC-1 and PC-2 scores. The number of clusters was determined visually, by plotting agglomeration coefficients against clustering stage. Clusters combined in stages preceding the steepest increase in coefficient value (which represents a significant information gain, or, between-case dissimilarly) were not recognized.

## 5.4 Results

In all five PCA performed (surface-layer *Calanus hyperboreus* & *C. glacialis*; deep-layer *C. hyperboreus*, *C. glacialis* & *Metridia longa*; Figures 5.2-5.6), principal components (PC) 1 and 2 accounted for 51 to 54% of the total variance in copepod lipid content and composition. In surface-layer samples of *Calanus hyperboreus*, several diatom, phytoplankton, and herbivory biomarkers (16:2(n-4), 16:4(n-1), 20:5(n-3),

ΣPUFA, UC, (n-3)/(n-6)) were negatively correlated with PC-1 (Figure 5.2a). Conversely, lipids with positive PC-1 correlations were mainly saturated (SFA) and monounsaturated (MUFA) fatty acids (14:0, 16:0, 18:0, ΣSFA, ΣMUFA), including copepod (18:1(n-9), 20:1(n-9), 22:1(n-11)) and dinoflagellate (18:4(n-3), DHA/EPA) biomarkers. PC-1 thus represents a separation of phytoplankton/herbivorous and animal/carnivorous lipid. PC-2 separated diatom from dinoflagellate biomarkers. 16:1(n-7), 20:3(n-6), and 16:1(n-7)/16:0 were positively correlated with PC-2, while 22:6(n-3) was negatively correlated with PC-2.

Cluster analysis of scores revealed four groups of stations characterized by distinct lipid compositions (Figure 5.2b). Interpretation of PC-1 and PC-2 suggest that surface-layer *Calanus hyperboreus* at Stations 01, 03, 14, 32, 35, 44, 45, 54b and 68 were feeding herbivorously on diatoms, while copepods at Stations 06, 40 and 66 were feeding herbivorously on dinoflagellates. Thus copepods from Stations 50, 54a and 58 were presumably feeding omnivorously, with animals from the latter two stations having eaten more dinoflagellates and fewer diatoms. The raw lipid data reflect these classifications. Surface-layer *C. hyperboreus* from Stations 50, 54a and 58 contained lower relative amounts of 16:4(n-1), 20:5(n-3), ΣPUFA and had lower UC and (n-3)/(n-6) ratios than animals from almost all other stations (Table 5.2). Animals from Stations 06, 40, 54a, 58 and 66 were characterized by low 16:1(n-7)/16:0 ratios. Furthermore, those at Stations

54a and 58 had high proportions of 18:4(n-3) and 22:6(n-3) and elevated DHA/EPA ratios.

For samples of *Calanus hyperboreus* from the deep layer, several diatom (16:3(n-4), 16:4(n-1), 20:5(n-3), 16:1(n-7)/16:0) and herbivory ((n-3)/(n-6)) indices were negatively correlated with PC-1 (Figure 5.3a). On the other hand, $C_{18}$ PUFA (18:2(n-6), 18:3(n-3), 18:4(n-3)), DHA/EPA ratios and bacterial markers (15:0, 18:1(n-7), ΣOBFA) were characterized by high positive PC-1 correlations. PC-2 separated copepod fatty acids from bacterial ones: 20:1(n-7), 22:1(n-7), TFA (total fatty acids) and ΣMUFA were negatively correlated with PC-2 while 15:1 and ΣOBFA were positively correlated with PC-2.

Based on this information and the results of the CA of station scores (Figure 5.3b), deep-layer *Calanus hyperboreus* at Stations 02, 03, 06, 14, 32, 35, 45, 54a, 54b and 66 had probably consumed diatoms. Copepods from Station 54b may have eaten bacteria (presumably indirectly as marine snow or bacterivorous microzooplankton) in addition to diatoms, and animals at Stations 35 and 66 were possibly in early diapause. Raw data show that proportions of 20:1(n-7), 22:1(n-7), ΣMUFA as well as total lipid (TL) and fatty acid content were highest in animals from Stations 35 and 66 (Table 5.3). Animals at Stations 58 and 76 were feeding omnivorously with a dinoflagellate and bacterial component in their diet. The raw data confirm that copepods from Stations 54b, 58 and

76 had slightly higher relative amounts of OBFA and those at Stations 58 and 76 had elevated levels of 18:4(n-3), 22:6(n-3) and very high DHA/EPA ratios.

In surface-layer *Calanus glacialis* samples, PC-1 separated diatom, phytoplankton and herbivory markers (16:4(n-1), 20:5(n-3), ΣPUFA, UC, (n-3)/(n-6); negative scores) from copepod (18:1(n-9), 20:1(n-9)), bacterial (15:0, i-17:0, 18:1(n-7), ΣOBFA) and dinoflagellate (18:4(n-3)) markers (Figure 5.4a). PC-2 separated dinoflagellate (22:6(n-3), DHA/EPA) from bacterial biomarkers (ai-17:0). The classification of scores by CA revealed five station groups (Figure 5.4b). PC-1 and PC-2 indicate that *C. glacialis* fed mostly herbivorously on either diatoms (Stations 01, 03, 06, 32, 35, 40, 44, 45, 54b, 66, 68) or dinoflagellates (Station 14). Animals feeding on diatoms may have ingested bacteria as well (especially at Station 44). Copepods at Stations 54a and 76 were likely feeding omnivorously with a substantial dinoflagellate and small bacterial input in their diet. The raw data show that UC and (n-3)/(n-6) ratios were lowest at Stations 54a and 76 and relative contributions of 16:4(n-1) and 20:5(n-3) were much reduced compared to all other stations (Table 5.4). Copepods from these two stations and those at Station 14 generally had elevated DHA/EPA ratios and high proportions of 18:4(n-3) and 22:6(n-3). Relative proportions of OBFA were low at Station 14 and highest at Stations 44 and 54a.

PC-1 separated diatom (16:4(n-1), 20:5(n-3)), phytoplankton (ΣPUFA) and herbivory (UC, (n-3)/(n-6)) markers from copepod (18:1(n-9), 20:1(n-11), WE), dinoflagellate (18:4(n-3), DHA/EPA) and bacterial (15:0) markers in deep samples of *Calanus glacialis* (Figure 5.5a). PC-2 was negatively correlated with TL and positively correlated with a minor 16-carbon PUFA (16:4(n-3)), two bacterial fatty acids (i-17:0, ai-17:0) and ΣOBFA. According to the CA, the station scores fell into four main groups (Figure 5.5b). Therefore, it was deduced that copepods from most stations were feeding herbivorously on diatoms (02, 03, 06, 14, 32, 35, 45, 54a, 54b, 66) and those with high PC-2 scores may have also eaten bacteria (02, 32). Animals at Stations 58 and 76 were omnivorous and had probably ingested significant amounts of dinoflagellates. Samples with negative PC-2 scores (03, 06, 14, 35, 66) were possibly in early diapause due to their higher TL content (Table 5.5). Copepods from Stations 58 and 76 had lower UC and 16:4(n-1) and 20:5(n-3) levels compared to all other stations. Also, copepods at these stations were generally characterized by higher proportions of 18:4(n-3) and 22:6(n-3) and elevated DHA/EPA ratios.

For deep-layer samples of *Metridia longa*, PC-1 represented a separation of copepod health indices (TFA, TL, % lipid, WE, WE/DM (DM=dry mass)) and variables associated with phytoplankton (UC, 20:4(n-3)) from bacterial biomarkers (15:0, 15:1, 18:1(n-7), ΣOBFA) (Figure 5.6a). PC-2 separated diatom (16:1(n-7), 16:3(n-4), 16:4(n-1), 16:1(n-7)/16:0) from dinoflagellate biomarkers ($C_{18}$ PUFA, 22:6(n-3),

DHA/EPA). Based on these distinctions and the grouping of station scores by CA (Figure 5.6b), copepods from Station 58 were lipid-poor omnivores with a bacterial component in their diet. Copepods from Stations 01, 02, 03, 06, 14, 32, 35, 40, 45, 54a and 54b may have been lipid-rich animals that were feeding herbivorously on diatoms. Animals at Stations 66 and 76 had likely ingested large proportions of dinoflagellates. The omnivorous copepods (Station 58) were characterized by low lipid levels (TFA, TL, WE/DM, % Lipid) and high proportions of OBFA (Table 5.6). Copepods at this station, as well as those at Stations 66 and 76, had very high DHA/EPA ratios and elevated proportions of 22:6(n-3) compared to animals at other stations.

The principal components and cluster analyses identified a discrete geographical region where omnivory and dinoflagellate consumption appeared to be important processes in all three species – the southeast (SE) corner of the polynya (Tables 5.7 & 5.8; Figure 5.1). However, at some SE stations all copepods fed herbivorously (54b, 68). For the two *Calanus* species, diapause was associated with prior diatom consumption and not with a recent history of omnivory or ingestion of dinoflagellates. In all species, ingestion of bacteria was common at "omnivory" stations, but it also occurred at stations where diatoms formed a large portion of the diet (mainly in *C. glacialis*).

The lipid composition of the seston was notably different at northwest (NW) and SE stations as well (Table 5.9). Considering both sampling depths, relative proportions of

16:1(n-7), 16:4(n-1), 20:5(n-3), and 16:1(n-7)/16:0 ratios were usually lower at SE stations. SE stations were also characterized by higher proportions of 18:4(n-3), 22:6(n-3) and ΣOBFA, and higher dinoflagellate indices (i.e., $C_{18}$PUFA+$C_{22}$PUFA; DHA/EPA). Furthermore, absolute amounts of TL, TFA and PUFA were lower at SE stations as compared to those in the NW. Considering only the 1% light depth, SE stations had lower levels of particulate organic carbon (POC), autotrophs and the diatom *Chaetoceros socialis*, as well as lower overall rates of primary production (total, > 5μm, < 5μm) (Table 5.10). Stations in the SE were also characterized by higher carbon to chlorophyll ratios (C:Chl), more autotrophic dinoflagellates and higher bacterial biomass.

## 5.5 Discussion

Stations identified as southeastern (50, 54a, 54b, 58, 66, 68, 76: Figure 5.1) are under the same physical control: all are influenced by a north-moving branch of the West Greenland Current (WGC). SE stations are part of the "Southern Assembly" (SA) or "Southern-North Water Assembly" (S-NWA) as defined by Bâcle et al. (2002). SA stations are entirely influenced by the WGC that carries water from the Labrador Sea and Baffin Bay northward along the western coast of Greenland. The "North Water Assembly" results from the mixing of arctic water (from Smith and Jones Sounds) and the WGC; those in the south (i.e., S-NWA) are characterized by the strongest presence of Atlantic water. Depending on the sampling month, Station 50 was sometimes considered a "Central-NWA" station by Bâcle et al. (2002) because Atlantic water was not always

detected. Except for very early in the year (e.g., April), the WGC is much poorer in nitrate and silicate than is arctic water (Tremblay et al. 2002).

Lovejoy et al. (2002a) found that microplankton assemblages were strongly related to water mass gradients within the North Water. During July 1998 (temporally closest to my data), ~70% of the microplankton biomass at 1% light in Smith Sound (e.g., Stations 01, 02, 03, 06, 14) was diatoms, while at central (e.g., 32, 35, 44, 45) and Carey Island stations (e.g., 40, 54) diatoms constituted ~30% of the biomass. Diatom contributions were lowest (~10%) in the WGC region (Stations 68, 76). In all cases, the remaining microplankton was composed of varying proportions of ciliates, flagellates and dinoflagellates, with the highest ciliate contributions at SE stations. These results corroborate the seston data (Tables 5.9 & 5.10) and demonstrate that towards the end of the open water season, the microbial loop was more dominant at SE than NW stations. These spatial differences may be related in part to the nutrient characteristics of the water supplying the SE region.

Despite the localized microbial conditions, copepod diets were not uniform within the SE region. Animals at Stations 54b, 68 (all species) and 66 (two *Calanus* species) were feeding herbivorously at the time of sampling. Based on the seston data, copepods from all SE stations were expected to be feeding omnivorously. However, a fall diatom bloom may have been occurring at Station 54b. Compared to Station 54a, sampled 12

days earlier. Station 54b was characterized by primary productivity, chlorophyll and total seston lipid levels roughly two-fold higher (data not shown). Moreover, between 07 September (54a) and 19 September (54b), the 1% light level decreased from 39 to 23 m. High nutrient levels were observed at Station 54a at 1% light ($NO_3$+$NO_2$=13.5 μM) and by the time 54b was sampled, they were completely depleted (0.8 μM). Presumably this station experienced a localized pulse of nutrients that created favorable diatom bloom conditions. In addition to WGC input, Stations 66 and 68 may have been influenced by water originating from Jones Sound. Lovejoy et al. (2002a) found that in June 1998, nearby Station 64 (75°15'N, 77°08'W) had a distinct microplankton assemblage, characterized by high proportions of ribbon-forming pennate diatoms, that was attributed to Jones Sound outflow. An input of silicate-rich Arctic water could have caused a short-lived transient bloom at Stations 66 and 68, although there is no specific evidence to support this suggestion.

These observations indicate that copepods responded to both large (NW *vs.* SE) and small-scale (54a *vs.* 54b) changes in microplankton assemblages. This is in agreement with other studies conducted in polar oceans, in which copepod lipids closely mirrored available prey (Kattner et al. 1989; Cripps and Hill 1998). Copepods in the NOW thus appeared to feed on the most abundant prey, including diatoms, dinoflagellates and microbial material. The highly varied diet observed may relate to the continuous presence of suitable prey throughout the sampling region. Lovejoy et al.

(2002b) found that both protist and diatom communities in the polynya were characterized by a prolonged dominance of large cells (> 20 μm). Copepods usually prefer larger cells when offered a choice (Mullin 1963; Frost 1977). This led Lovejoy et al. to speculate that the constant availability of optimal prey for zooplankton contributes to the overall productivity of the polynya.

Similar spatial patterns in copepod feeding strategy were observed in both surface and deep layers. PCA and CA revealed that at "omnivory" stations (Tables 5.7 & 5.8), copepods often fed omnivorously in both layers. This may mean that copepods from the entire water column were feeding on the same food source, probably that derived from the chlorophyll maximum. Food was much more plentiful (higher TL, TFA, PUFA levels) and nutritious (higher %PUFA) at 1% light than at depth (Table 5.9). Animals in deep strata at some stations may have stopped feeding and entered diapause. By autumn in the North Water, *Calanus hyperboreus* and *C. glacialis* no longer produce eggs (Ringuette et al. 2002), which suggests that their active feeding season was coming to a close. Thus, lipid signals in animals at depth may reflect food ingested weeks ago in shallow water. Alternatively, animals in the deep layer could have been actively feeding via vertical migration or on sinking material.

The multivariate analyses suggest that bacteria formed part of the diet of all copepods investigated, but to varying extents. In *Calanus hyperboreus* and *Metridia*

*longa*, ingestion of bacteria occurred only at SE stations where bacterial biomass was highest. *C. glacialis*, on the other hand, appears to have ingested bacteria in both the SE and NW, often in association with diatom-based herbivory. A moderately strong linear relationship between seston and copepod OBFA concentration was found for this species but not the other two (data not shown). Also, Stevens et al. (in press 'b') found that relative OBFA contributions were often higher in *C. glacialis* than in *C. hyperboreus* and *M. longa*. Compared to the other copepods, *C. glacialis* may be more likely to eat dying cells or marine snow, where bacterial and diatom biomarkers presumably co-occur. At SE stations, all species may have ingested bacterivorous protists.

Considering the variable loadings, UC, (n-3)/(n-6), and ΣPUFA were always associated with diatom biomarkers. Conversely, microbial markers like ΣOBFA and 18:1(n-7) were always associated with dinoflagellate and carnivory indices. PCA was therefore very useful in separating microbial biomarkers from those associated with the classical food web; there was rarely any overlap. The specific variable groupings suggest that PUFA content and UC, and hence herbivory, were dependent on diatom production and not on other autotrophic microplankton (e.g., dinoflagellates). As expected, copepod omnivory in the polynya was inversely related to the availability of diatoms.

## 5.6 Conclusions

Results indicate that herbivory is an important process in the NOW, even at the end of the open water season, and is strongly linked to the prolonged diatom blooms that characterize this productive region in the fall. Copepods fed omnivorously only at stations in the SE where the microbial loop was predominant, but even in this region, they quickly responded to localized diatom bloom events. In all samples analyzed, the essential fatty acids EPA and DHA were abundant, although relative levels changed with diet and feeding strategy. Copepods feeding both herbivorously and omnivorously represent nutritious sources of food for higher order consumers in the NOW.

## 5.7 Acknowledgements

The author would like to thank the officers, crew and captain of the *N.G.C.C. Pierre Radisson*, as well as M. Fortier, N. Kobayashi, M. Ringuette, M. Sampei, P. Sirois and K. Takahashi for assistance in the field. Funding for this project was provided by an NSERC grant awarded to D. Deibel, R. Rivkin and M. Anderson, the NSERC Discovery Grant of D. Deibel, and a Northern Scientific Training Program grant awarded to C. Stevens.

## 5.8 References

Ackman RG, Tocher CS (1968) Marine phytoplankter fatty acids. J Fish Res Board Can 25:1603-1620

Ackman RG (1986) WCOT (Capillary) Gas-liquid chromatography. In: Hamilton RJ, Rossell JB (eds) Analysis of Oils and Fats. Elsevier, London, pp 137-206

Acuña JL, Deibel D, Saunders PA, Booth B, Hatfield E, Klein B, Mei Z-P, Rivkin R (2002) Phytoplankton ingestion by appendicularians in the North Water. Deep-Sea Res II 49:5101-5115

Albers CS, Kattner G, Hagen W (1996) The compositions of wax esters, triacylglycerols and phospholipids in Arctic and Antarctic copepods: evidence of energetic adaptations. Mar Chem 55:347-358

Atkinson A (1996) Subantarctic copepods in an oceanic, low chlorophyll environment: ciliate predation, food selectivity and impact on prey populations. Mar Ecol Prog Ser 130:85-96

Auel H, Harjes M, de Rocha R, Stübing D, Hagen W (2002) Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperiid amphipods *Themisto abyssorum* and *T. libellulua*. Polar Biol 25:374-383

Bâcle J, Carmack EC, Ingram RG (2002) Water column structure and circulation under the North Water during spring transition: April-July 1998. Deep-Sea Res II 49:4907-4925

Booth BC, Larouche P, Bélanger S, Klein B, Amiel D, Mei Z-P (2002) Dynamics of *Chaetoceros socials* blooms in the North Water. Deep-Sea Res II 49:5003-5025

Brett MT, Müller-Navarra DC (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshw Biol 38:483-499

Budge SM, Parrish CC (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29:1547-1559

Colombo JC, Silverberg N, Gearing JN (1996) Lipid biogeochemistry in the Laurentian Trough: I-fatty acids, sterols and aliphatic hydrocarbons in rapidly settling particles. Org Geochem 25:211-225

Cripps GC, Hill HJ (1998) Changes in lipid composition of copepods and *Euphausia superba* associated with diet and environmental conditions in the marginal ice zone, Bellingshausen Sea, Antarctica. Deep-Sea Res I 45:1357-1381

Deming JW, Fortier L, Fukuchi M (2002) The International North Water Polynya Study (NOW): a brief overview. Deep-Sea Res II 49:4887-4892

DeMott WR, Müller-Navarra D (1997) The importance of highly unsaturated fatty acids in zooplankton nutrition: evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. Freshw Biol 38:649-664

Desvilettes Ch, Bourdier G, Breton JC, Combrouze Ph (1994) Fatty acids as organic markers for the study of trophic relationships in littoral cladoceran communities. J Plankton Res 16:643-659

Falk-Petersen S, Sargent JR, Tande KS (1987) Lipid composition of zooplankton in relation to the sub-arctic food web. Polar Biol 8:115-120

Falk-Petersen S, Hagen W, Kattner G, Clarke A, Sargent J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. Can J Fish Aquat Sci 57:178-191

Field JC, Clarke KR, Warwick RM (1982) A practical strategy for analyzing multispecies distribution patterns. Mar Ecol Prog Ser 8:37-52

Frost BW (1977) Feeding behavior of *Calanus pacificus* in mixtures of food particles. Limnol Oceanogr 22:472-491

Graeve M, Hagen W, Kattner G (1994) Herbivorous or omnivorous? On the significance of lipid compositions as trophic markers in Antarctic copepods. Deep-Sea Res I 41:915-924

Hobson KA, Fisk A, Karnovsky N, Holst M, Gagnon J-M, Fortier M (2002) A stable isotope ($d^{13}C$, $d^{15}C$) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. Deep-Sea Res II 49:5131-5150

Hopkins TL (1987) Midwater food web in McMurdo Sound, Ross Sea, Antarctica. Mar Biol 96:93-106

Hopkins TL, Torres JJ (1989) Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep-Sea Res I 36:543-560

Jónasdóttir SH, Fields D, Pantoja S (1995) Copepod egg production in Long Island Sound, USA, as a function of the chemical composition of seston. Mar Ecol Prog Ser 119:87-98

Kaneda T (1991) Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol Rev 55:288-302

Karnovsky NJ, Hunt GJ (2002) Estimation of carbon flux to dovekies (Alle alle) in the North Water. Deep-Sea Res II 49: 5117-5130

Kattner G, Hirche HJ, Krause M (1989) Spatial variability in lipid composition of calanoid copepods from Fram Strait, the Arctic. Mar Biol 102:473-480

Kattner G, Hagen W (1995) Polar herbivorous copepods - different pathways in lipid biosynthesis. ICES J Mar Sci 52:329-335

Klein B, LeBlanc B, Mei Z-P, Beret R, Michaud J, Mundy C-J, von Quillfeldt CH, Garneau M-È, Roy S, Gratton Y, Cochran JK, Bélanger S, Larouche P, Pakulski JD, Rivkin RB, Legendre L (2002) Phytoplankton biomass, production and potential export in the North Water. Deep-Sea Res II 49:4983-5002

Landry MR (1981) Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. Mar Biol 65:77-82

Lee RF, Nevenzel JC, Paffenhöfer G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9:99-108

Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in arctic marine ecosystems. Mar Ecol Prog Ser 204:65-77

Lovejoy C, Legendre L, Martineau M-J, Bâcle J, von Quillfeldt CH (2002a) Distribution of phytoplankton and other protists in the North Water. Deep-Sea Res II 49:5027-5047

Lovejoy C, Legendre L, Price NM (2002b) Prolonged diatom blooms and microbial food web dynamics: experimental results from an Arctic polynya. Aquat Microb Ecol 29:267-278

Mayzaud P, Eaton CA, Ackman RG (1976) The occurrence and distribution of octadecapentaenoic acid in a natural plankton population. A possible food chain index. Lipids 11:858-862

Mees J, Hamerlynck O (1992) Spatial community structure of the winter hyperbenthos of the Schelde Estuary, the Netherlands, and the adjacent coastal waters. Neth J Sea Res 29:357-370

Meglen RR (1992) Examining large databases: a chemometric approach using principal component analysis. Mar Chem 39:217-237

Middleboe M, Nielson TG, Bjørnsen PK (2002) Viral and bacterial production in the North Water: *in situ* measurements, batch-culture experiments and characterization and distribution of a virus-host system. Deep-Sea Res II 49:5063-5079

Mullin MM (1963) Some factors affecting the feeding of marine copepods of the genus *Calanus*. Limnol Oceanogr 8:239-250

Nelson MM, Mooney BD, Nichols PD, Phleger CF (2001) Lipids of Antarctic Ocean amphipods: food chain interactions and the occurrence of novel biomarkers. Mar Chem 73:53-64

Ohman MD (1997) On the determination of zooplankton lipid content and the occurrence of gelatinous copepods. J Plankton Res 19:1235-1250

Parrish CC (1987) Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can J Fish Aquat Sci 44:722-731

Parrish CC (1999) Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC (eds) Lipids in Freshwater Ecosystems. Springer-Verlag, New York, pp 4-20

Pond DW, Bell MV, Harris RP, Sargent JR (1998) Microplanktonic polyunsaturated fatty acid markers: a mesocosm trial. Est Coast Shelf Sci 46:61-67

Pranal V, Fiala-Médioni A, Guezennec J (1996) Fatty acid characteristics in two symbiotic gastropods from a deep hydrothermal vent of the West Pacific. Mar Ecol Prog Ser 142:175-184

Ringuette M, Fortier L, Fortier M, Runge JA, Bélanger S, Larouche P, Weslawski J-M, Kwasniewski S (2002) Advanced recruitment and accelerated population development in Arctic calanoid copepods of the North Water. Deep-Sea Res II 49:5081-5099

Sargent JR, Falk-Petersen S (1981) Ecological investigations on the zooplankton community in Balsfjorden, Northern Norway: Lipids and fatty acids in *Meganyctiphanes norvegica*, *Thysanoessa raschi* and *T. inermis* during mid-winter. Mar Biol 62:131-137

Sargent JR, Eilersten HC, Falk-Petersen S, Taasen JP (1985) Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. Mar Biol 85:109-116

Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleig MA (ed) Microbes in the Sea. Ellis Harwood, Chichester, p 119-138

Scott CL, Falk-Petersen S, Sargent JR, Hop H, Lønne OJ, Poltermann M (1999) Lipids and trophic interactions of ice fauna and pelagic zooplankton in the marginal ice zone of the Barents Sea. Polar Biol 21:65-70

Stevens CJ, Deibel D, Parrish CC. (a) Incorporation of bacterial fatty acids and changes in a wax ester-based omnivory index during a long-term incubation experiment with *Calanus glacialis* Jaschnov. J Exp Mar Biol Ecol (in press).

Stevens CJ, Deibel D, Parrish CC. (b) Species-specific differences in lipid composition and omnivory indices in arctic copepods collected in deep water during autumn (North Water Polynya). Mar Biol (in press).

Tremblay J-E, Gratton Y, Fauchot J, Price NM (2002) Climatic and oceanic forcing of new, net, and diatom production in the North Water. Deep-Sea Res II 49:4927-4946

Viso A-C, Marty J-C (1993) Fatty acids from 28 marine microalgae. Phytochemistry 34:1521-1533

Volkman JK, Jeffrey SW, Nichols PD, Rogers GI, Garland CD (1989) Fatty acid and lipid composition of 10 species of microalgae used in mariculture. J Exp Mar Biol Ecol 128:219-240

Weers PMM, Siewertsen K, Gulati RD (1997) Is the fatty acid composition of *Daphnia galeata* determined by the fatty acid composition of the ingested diet? Freshw Biol 38:731-738

Yunker MB, Macdonald RW, Veltkamp DJ, Cretney WJ (1995) Terrestrial and marine biomarkers in a seasonally ice-covered Arctic estuary - integration of multivariate and biomarker approaches. Mar Chem 49:1-50

## 5.9 Tables

Table 5.1. Descriptions of stations sampled for lipids in the North Water Polynya.

| Station | Date (1999) | $Z_{1\%}$ (m) | $Z_m$ (m) | Copepod Samples | | Seston Samples |
|---|---|---|---|---|---|---|
| | | | | Depth (m) | Description[a] | Depth (m) |
| 01 | 02-03 Sept | 20 | 18 | 75-0 | Ch, Cg | 12 |
| | | | | 150-0 | Ml | 100 |
| 02 | 11 Sept | 20 | 9 | 50-0 | ncp | - |
| | | | | 250-50 | Ch*, Cg*, Ml | 200 |
| 03 | 04 Sept | 23 | 12 | 60-0 | Ch, Cg | 15* |
| | | | | 250-60 | Ch, Cg, Ml* | 200 |
| 06 | 13 Sept | 23 | 14 | 75-0 | Ch, Cg | 20 |
| | | | | 150-75 | Ch, Cg, Ml | 200 |
| 14 | 25 Sept | 34 | 12 | 100-0 | Ch*, Cg, Ml | 25 |
| | | | | 300-100 | Ch, Cg, Ml | 200 |
| 32 | 22 Sept | 42 | 17 | 75-0 | Ch, Cg | 42* |
| | | | | 195-75 | Ch, Cg*, Ml | 100 |
| 35 | 01 Sept | 15 | 13 | 60-0 | Ch, Cg | 15 |
| | | | | 250-60 | Ch, Cg, Ml | 200 |
| 40 | 05-06 Sept | 24 | 9 | 60-0 | Ch, Cg | 24 |
| | | | | 250-0 | Ml | 200 |
| 44 | 30 Aug | 49 | 12 | 75-0 | Ch, Cg | - |
| 45 | 17-18 Sept | 35 | 20 | 75-0 | Ch, Cg, Ml | 29 |
| | | | | 200-75 | Ch, Cg, Ml | 150 |
| 50 | 29 Aug | 34 | 14 | 75-0 | Ch | - |
| 50 | 10 Sept | 22 | 12 | - | - | 22* |
| 54 | 07 Sept | 39 | 7 | 75-0 | Ch, Cg | 39 |
| | | | | 250-75 | Ch, Cg, Ml* | 200 |
| 54 | 19 Sept | 23 | 18 | 75-0 | Ch, Cg* | 23 |
| | | | | 250-75 | Ch, Cg, Ml | 200 |
| 58 | 08 Sept | 28 | 6 | 50-0 | Ch | 29* |
| | | | | 150-50 | Ch, Cg, Ml | 100 |
| 66 | 29-30 Sept | 42 | 11 | 75-0 | Ch, Cg, Ml* | 35 |
| | | | | 250-75 | Ch, Cg, Ml | 200 |
| 68 | 27 Aug | 33 | 18 | 100-0 | Ch, Cg | 33 |
| 76 | 01 Oct | 32 | 18 | 75-0 | Cg, Ml | 32 |
| | | | | 250-75 | Ch*, Cg, Ml | 200 |

[a]Ch=*Calanus hyperboreus* CV; Cg=*Calanus glacialis* CV; Ml=*Metridia longa* female; ncp=no copepods present; *3 replicate samples taken; $Z_{1\%}$=1% light depth (data provided by B. Klein); $Z_m$=mixed layer depth (data provided by Y. Gratton)

Table 5.2. Variables with significant PCA loadings in surface-layer *Calanus hyperboreus* CV collected in the North Water during autumn 1999.

| Lipid[a] (%) | 01 | 03 | 06 | 14 | 32 | 35 | 40 | 44 | 45 | *50 | *54a | *54b | *58 | *66 | *68 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 2.9 | 2.9 | 2.7 | 2.4 | 2.7 | 3.0 | 2.6 | 2.7 | 2.6 | 4.2 | 4.3 | 2.7 | 3.5 | 2.6 | 3.3 |
| 15:0 | 0.1 | tr | nd | 0.1 | 0.1 | 0.2 | tr | 0.1 | 0.1 | 0.2 | 0.3 | nd | 0.2 | 0.1 | 0.1 |
| 16:0 | 3.2 | 2.6 | 2.3 | 2.3 | 2.5 | 3.0 | 2.3 | 2.4 | 2.6 | 3.9 | 5.0 | 2.5 | 4.3 | 2.8 | 3.2 |
| 16:1(n-7) | 23.6 | 19.7 | 11.7 | 16.8 | 18.4 | 20.5 | 13.0 | 19.0 | 20.0 | 29.7 | 13.8 | 16.8 | 11.1 | 13.8 | 19.9 |
| 16:1(n-5) | 0.3 | 0.2 | 0.1 | 0.2 | 0.2 | 0.5 | 0.2 | 0.2 | 0.2 | 0.2 | 0.5 | 0.2 | 0.5 | 0.2 | 0.3 |
| 16:2(n-4) | 1.6 | 1.5 | 1.6 | 1.8 | 1.5 | 1.8 | 1.6 | 1.5 | 1.7 | 1.5 | 1.2 | 1.8 | 1.3 | 1.6 | 1.4 |
| 16:4(n-1) | 5.1 | 4.7 | 7.2 | 6.8 | 5.6 | 5.7 | 6.0 | 4.9 | 6.1 | 2.7 | 2.3 | 5.1 | 1.6 | 5.2 | 4.0 |
| 18:0 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.6 | 0.7 | 0.1 | 0.4 | 0.5 | 0.2 |
| 18:1(n-9) | 2.0 | 1.4 | 1.0 | 1.6 | 1.5 | 1.7 | 1.3 | 1.6 | 1.6 | 2.0 | 3.0 | 1.6 | 3.9 | 1.5 | 2.3 |
| 18:1(n-7) | 1.3 | 1.2 | 0.9 | 1.2 | 1.2 | 1.1 | 1.0 | 1.5 | 1.1 | 1.6 | 1.3 | 1.2 | 1.4 | 1.1 | 1.3 |
| 18:1(n-5) | 0.5 | 0.4 | 0.4 | tr | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 | 0.5 | 0.7 | 0.4 | 0.7 | 0.4 | 0.5 |
| 18:2(n-6) | 0.6 | 0.6 | 0.4 | 0.5 | 0.5 | 0.6 | 0.5 | 0.7 | 0.5 | 0.7 | 2.0 | 0.6 | 3.1 | 0.5 | 1.3 |
| 18:3(n-6) | 0.5 | 0.4 | 0.2 | 0.3 | 0.2 | 0.3 | 0.2 | 0.3 | 0.3 | 0.7 | 0.2 | 0.3 | 0.3 | 0.4 | 0.3 |
| 18:3(n-3) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 1.1 | 0.2 | 1.9 | 0.6 | 0.6 |
| 18:4(n-3) | 1.9 | 1.3 | 1.1 | 1.7 | 1.4 | 1.6 | 2.0 | 0.1 | 1.7 | 1.3 | 7.5 | 2.2 | 9.7 | 1.7 | 4.6 |
| 20:1(n-9) | 8.1 | 6.2 | 4.7 | 7.3 | 5.5 | 7.0 | 6.0 | 4.7 | 7.5 | 6.9 | 10.0 | 7.2 | 10.2 | 5.6 | 9.0 |
| 20:2(n-6) | 0.2 | tr | nd | nd | tr | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.3 | nd | 0.2 |
| 20:3(n-6) | 0.2 | 0.1 | tr | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.6 | nd | 0.2 | nd | nd | nd |
| 20:5(n-3) | 26.1 | 33.7 | 42.7 | 35.2 | 34.8 | 30.9 | 42.1 | 39.6 | 32.3 | 20.0 | 16.8 | 33.2 | 12.4 | 30.3 | 25.3 |
| 22:1(n-11) | 6.6 | 6.6 | 4.1 | 7.1 | 5.8 | 5.9 | 5.4 | 4.4 | 5.6 | 8.5 | 8.4 | 7.9 | 13.0 | 5.2 | 7.2 |
| 22:1(n-9) | 1.3 | 0.9 | 0.7 | 1.2 | 0.8 | 1.1 | 1.4 | 1.1 | 1.5 | 1.2 | 1.8 | 2.0 | 2.4 | 0.8 | 1.5 |
| 22:1(n-7) | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.5 | 0.3 | 0.2 | 0.4 | 0.1 | 0.2 |
| 22:6(n-3) | 3.1 | 6.1 | 5.6 | 3.4 | 7.2 | 3.0 | 4.2 | 3.6 | 3.0 | 2.6 | 7.4 | 4.2 | 9.7 | 9.9 | 4.6 |
| ΣOBFA | 0.8 | 0.5 | 1.1 | 0.7 | 0.6 | 1.5 | 0.7 | 0.9 | 1.3 | 0.7 | 1.1 | 0.8 | 1.0 | 0.6 | 0.9 |
| ΣSFA | 4.2 | 3.2 | 3.5 | 3.1 | 3.3 | 4.3 | 3.2 | 3.3 | 3.8 | 6.8 | 9.5 | 3.2 | 5.7 | 4.2 | 4.3 |
| ΣMUFA | 45.6 | 37.9 | 25.0 | 36.6 | 35.4 | 40.4 | 29.8 | 34.3 | 39.8 | 53.8 | 41.2 | 38.7 | 45.2 | 30.1 | 43.7 |
| ΣPUFA | 47.1 | 55.5 | 68.7 | 54.0 | 57.7 | 52.0 | 64.0 | 59.5 | 54.8 | 34.5 | 44.4 | 55.0 | 45.3 | 63.1 | 48.4 |
| 16:1/16:0 | 7.3 | 7.6 | 5.0 | 7.2 | 7.2 | 6.8 | 5.7 | 7.8 | 7.6 | 7.6 | 2.7 | 6.7 | 2.6 | 4.9 | 6.2 |
| DHA/EPA | 0.12 | 0.18 | 0.13 | 0.10 | 0.21 | 0.10 | 0.10 | 0.09 | 0.09 | 0.13 | 0.44 | 0.13 | 0.78 | 0.33 | 0.18 |
| (n-3)/(n-6) | 15.3 | 28.1 | 55.9 | 34.2 | 34.8 | 22.8 | 33.7 | 23.0 | 28.2 | 12.1 | 12.8 | 24.1 | 7.6 | 56.4 | 9.1 |
| UC | 0.44 | 0.53 | 0.67 | 0.52 | 0.55 | 0.53 | 0.69 | 0.58 | 0.55 | 0.30 | 0.47 | -- | 0.40 | 0.50 | 0.46 |

[a]all data % or ratios; column headings are North Water station numbers; *SE stations; 16:1=16:1(n-7); DHA=docosahexaenoic acid (22:6(n-3)); EPA=eicosapentaenoic acid (20:5(n-3)); nd=not detected; tr=trace (<0.1%))

Table 5.3. Lipid variables with significant PCA loadings in deep-layer *Calanus hyperboreus* CV collected in the North Water during autumn 1999.

| Lipid (%) | 02 | 03 | 06 | 14 | 32 | 35 | 45 | *54a | *54b | *58 | *66 | *76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 2.9 | 2.8 | 3.0 | 2.5 | 2.8 | 3.8 | 3.2 | 3.0 | 3.2 | 4.6 | 3.3 | 3.5 |
| 15:0 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.1 | 0.2 |
| 15:1 | 0.1 | 0.1 | 0.1 | tr | 0.1 | nd | 0.1 | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 |
| 16:0 | 2.8 | 3.2 | 2.9 | 2.5 | 2.5 | 3.3 | 2.9 | 2.6 | 3.6 | 5.3 | 3.5 | 3.7 |
| 16:1(n-5) | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.4 | 0.2 | 0.2 | 0.4 | 0.6 | 0.2 | 0.6 |
| 16:3(n-4) | 1.7 | 2.0 | 1.5 | 1.7 | 1.5 | 1.3 | 1.3 | 1.5 | 2.1 | 0.7 | 1.4 | 0.7 |
| 16:4(n-1) | 5.5 | 5.3 | 4.7 | 4.5 | 5.1 | 2.5 | 4.5 | 3.4 | 5.7 | 1.6 | 2.8 | 0.7 |
| 18:1(n-9) | 2.4 | 1.9 | 1.7 | 1.8 | 1.7 | 1.9 | 2.8 | 1.8 | 1.8 | 3.9 | 2.0 | 3.4 |
| 18:1(n-7) | 1.3 | 1.2 | 1.3 | 1.0 | 1.2 | 1.4 | 1.5 | 1.2 | 1.1 | 1.8 | 1.4 | 1.3 |
| 18:1(n-5) | 0.5 | 0.6 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.4 | 0.6 | 1.0 | 0.5 | 0.7 |
| 18:2(n-6) | 0.6 | 0.6 | 0.6 | 0.7 | 0.7 | 1.2 | 0.7 | 0.9 | 1.0 | 3.5 | 0.9 | 2.3 |
| 18:3(n-3) | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.4 | 0.2 | 0.3 | 0.3 | 1.6 | 0.3 | 1.7 |
| 18:4(n-3) | 1.9 | 1.9 | 1.7 | 1.6 | 1.3 | 4.8 | 2.0 | 3.8 | 2.4 | 10.1 | 2.6 | 11.5 |
| 18:4(n-1) | 0.6 | 0.7 | 0.6 | 0.5 | 0.6 | 0.5 | 0.6 | 0.5 | 0.9 | 0.4 | 0.4 | 0.3 |
| 20:1(n-7) | 1.2 | 0.9 | 1.5 | 1.2 | 1.0 | 1.8 | 1.5 | 1.1 | 0.9 | 0.2 | 1.8 | 1.1 |
| 20:2(n-6) | 0.1 | tr | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.4 | 0.1 | 0.3 |
| 20:3(n-6) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | nd | 0.1 | 0.1 |
| 20:5(n-3) | 27.5 | 24.4 | 27.6 | 26.1 | 31.1 | 20.2 | 25.1 | 26.2 | 30.0 | 10.9 | 17.5 | 10.6 |
| 22:1(n-7) | 0.3 | 0.2 | 0.3 | 0.3 | 0.2 | 0.4 | 0.3 | 0.3 | nd | 0.2 | 0.3 | 0.2 |
| 22:5(n-3) | 1.9 | 1.9 | 1.9 | 1.7 | 2.5 | 1.5 | 1.6 | 2.1 | 1.8 | 1.1 | 2.8 | 1.0 |
| ΣOBFA | 0.8 | 0.6 | 0.7 | 0.8 | 0.8 | 0.5 | 0.7 | 0.5 | 1.1 | 1.6 | 0.5 | 0.9 |
| ΣSFA | 7.1 | 6.7 | 6.7 | 6.3 | 6.1 | 8.3 | 6.8 | 6.9 | 8.2 | 12.2 | 7.9 | 8.3 |
| ΣMUFA | 43.4 | 43.5 | 44.6 | 43.9 | 38.6 | 48.9 | 49.5 | 41.2 | 38.5 | 44.5 | 50.3 | 47.0 |
| 16:1/16:0 | 7.6 | 7.1 | 7.6 | 8.0 | 8.2 | 8.7 | 9.0 | 7.6 | 6.7 | 4.3 | 8.2 | 4.6 |
| DHA/EPA | 0.16 | 0.30 | 0.20 | 0.30 | 0.24 | 0.27 | 0.12 | 0.22 | 0.13 | 0.72 | 0.47 | 1.02 |
| (n-3)/(n-6) | 17.5 | 16.8 | 25.3 | 21.2 | 27.1 | 11.9 | 19.4 | 22.5 | 20.5 | 7.3 | 15.2 | 10.9 |
| TFA[a] | 719.3 | 983.2 | 989.7 | 744.8 | 860.1 | 1118.6 | 794.9 | 485.4 | 438.3 | 453.8 | 1132.3 | 706.1 |
| UC | 0.51 | 0.34 | 0.46 | 0.41 | 0.46 | 0.34 | 0.38 | 0.46 | 0.44 | 0.30 | 0.29 | 0.37 |

[a]Total fatty acids (μg copepod$^{-1}$)

Table 5.4. Lipid variables with significant PCA loadings in surface-layer *Calanus glacialis* CV collected in the North Water during autumn 1999.

| Lipid (%) | 01 | 03 | 06 | 14 | 32 | 35 | 40 | 44 | 45 | *54a | *54b | *66 | *68 | *76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 8.2 | 7.9 | 7.8 | 7.0 | 8.1 | 8.9 | 7.6 | 9.1 | 8.2 | 10.4 | 7.7 | 8.4 | 8.3 | 9.5 |
| 14:1 | 0.1 | 0.3 | 0.3 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.4 | 0.1 | 0.2 | 0.2 | 0.5 |
| 15:0 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.8 | 0.2 | 0.2 | 0.3 | 0.4 |
| 16:0 | 5.8 | 5.3 | 5.5 | 5.6 | 5.9 | 5.7 | 4.9 | 6.2 | 5.5 | 8.5 | 5.9 | 5.6 | 6.5 | 8.3 |
| 16:1(n-5) | 0.4 | 0.3 | 0.3 | 0.3 | 0.4 | 0.5 | 0.4 | 0.5 | 0.5 | 0.8 | 0.4 | 0.4 | 0.4 | 0.7 |
| i-17:0 | 0.1 | nd | 0.1 | tr | tr | 0.1 | 0.1 | 0.2 | 0.1 | 0.3 | tr | 0.1 | nd | 0.1 |
| ai-17:0 | 0.4 | 0.3 | 0.4 | 0.3 | 0.3 | 0.5 | 0.6 | 0.7 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 | 0.2 |
| 16:4(n-1) | 5.2 | 5.8 | 7.0 | 5.6 | 4.9 | 5.4 | 5.2 | 5.0 | 4.7 | 2.1 | 5.2 | 5.8 | 5.4 | 1.2 |
| 18:1(n-9) | 2.1 | 1.6 | 1.6 | 1.6 | 1.4 | 1.7 | 1.8 | 1.9 | 1.6 | 4.6 | 1.9 | 1.7 | 2.4 | 4.5 |
| 18:1(n-7) | 0.9 | 0.5 | 0.6 | 0.6 | 0.7 | 0.8 | 0.6 | 0.8 | 0.7 | 1.0 | 0.8 | 0.7 | 0.7 | 0.9 |
| 18:2(n-6) | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 1.3 | 0.5 | 0.4 | 0.6 | 1.4 |
| 18:2(n-4) | 0.1 | nd | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.1 | 0.1 | tr | 0.6 |
| 18:3(n-3) | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.7 | 0.2 | 0.2 | 0.2 | 1.0 |
| 18:4(n-3) | 1.7 | 2.0 | 2.3 | 1.5 | 1.7 | 1.8 | 2.1 | 1.7 | 2.1 | 7.6 | 2.7 | 2.3 | 2.3 | 6.4 |
| 20:0 | 0.1 | tr | 0.1 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | nd | 0.1 | 0.3 | 0.1 | 0.3 |
| 20:1(n-9) | 8.7 | 8.6 | 6.6 | 4.6 | 5.2 | 6.9 | 6.9 | 6.8 | 5.6 | 9.4 | 7.2 | 6.3 | 7.0 | 12.5 |
| 20:4(n-3) | 0.3 | 0.4 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 0.3 | 0.7 | 0.4 | 0.4 | 0.4 | 0.7 |
| 20:5(n-3) | 27.0 | 29.8 | 32.7 | 29.3 | 33.1 | 35.2 | 36.9 | 30.4 | 33.5 | 10.6 | 32.2 | 31.3 | 31.6 | 9.9 |
| 22:6(n-3) | 2.1 | 3.9 | 6.6 | 11.0 | 5.6 | 2.7 | 4.6 | 2.3 | 3.1 | 3.5 | 5.4 | 5.7 | 3.3 | 6.1 |
| 24:1 | 0.3 | 0.6 | 0.7 | 1.6 | 0.8 | 0.3 | 0.3 | 0.2 | 0.3 | 0.4 | 0.3 | 1.6 | 0.2 | 1.1 |
| ΣOBFA | 1.1 | 0.7 | 1.0 | 1.0 | 1.1 | 1.3 | 1.9 | 2.2 | 1.6 | 3.9 | 1.1 | 1.3 | 1.5 | 1.6 |
| ΣSFA | 7.0 | 6.7 | 6.6 | 6.9 | 7.0 | 7.2 | 6.6 | 8.3 | 7.1 | 12.0 | 7.2 | 7.3 | 8.1 | 10.6 |
| ΣMUFA | 41.6 | 36.3 | 29.0 | 27.2 | 31.9 | 31.7 | 29.5 | 35.8 | 34.5 | 45.1 | 31.9 | 30.1 | 33.9 | 45.4 |
| ΣPUFA | 42.9 | 48.6 | 55.9 | 57.3 | 52.3 | 52.0 | 56.0 | 46.6 | 49.9 | 32.2 | 52.6 | 52.6 | 49.4 | 33.5 |
| DHA/EPA | 0.08 | 0.13 | 0.20 | 0.37 | 0.17 | 0.08 | 0.12 | 0.08 | 0.09 | 0.34 | 0.17 | 0.18 | 0.10 | 0.61 |
| (n-3)/(n-6) | 25.5 | 27.8 | 46.0 | 44.3 | 38.5 | 31.1 | 38.3 | 27.7 | 35.6 | 12.0 | 32.3 | 29.2 | 21.6 | 8.1 |
| WE | 84.7 | 81.1 | 83.6 | 75.0 | 76.8 | 84.7 | 84.7 | 76.1 | 82.9 | 90.6 | 87.5 | 81.8 | 81.3 | 89.4 |
| UC | 0.43 | 0.44 | 0.47 | 0.50 | 0.50 | 0.59 | 0.59 | 0.48 | 0.50 | 0.25 | 0.53 | 0.53 | 0.50 | 0.23 |

Table 5.5. Lipid variables with significant PCA loadings in deep-layer *Calanus glacialis* CV collected in the North Water during autumn 1999.

| Lipid (%) | 02 | 03 | 06 | 14 | 32 | 35 | 45 | *54a | *54b | *58 | *66 | *76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15:0 | 0.3 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.4 |
| 16:0 | 6.2 | 5.6 | 5.5 | 5.7 | 6.0 | 6.1 | 5.6 | 6.2 | 5.9 | 6.6 | 6.1 | 7.6 |
| i-17:0 | 0.1 | nd | tr | nd | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| ai-17:0 | 0.4 | 0.3 | 0.3 | 0.3 | 0.5 | 0.3 | 0.3 | 0.4 | 0.3 | 0.3 | 0.3 | 0.2 |
| 16:4(n-3) | 0.7 | 0.3 | 0.3 | 0.2 | 0.5 | 0.3 | 0.3 | 0.5 | 0.4 | 0.4 | 0.2 | 0.4 |
| 16:4(n-1) | 5.7 | 5.1 | 5.4 | 4.5 | 4.2 | 4.5 | 4.1 | 4.3 | 5.4 | 1.7 | 2.8 | 1.3 |
| 18:1(n-9) | 3.2 | 2.4 | 2.0 | 2.5 | 1.7 | 2.4 | 2.4 | 2.8 | 2.3 | 4.6 | 2.9 | 4.6 |
| 18:1(n-7) | 0.9 | 0.7 | 0.7 | 0.8 | 0.7 | 0.8 | 0.9 | 0.9 | 0.8 | 0.8 | 0.7 | 0.9 |
| 18:1(n-5) | 0.5 | 0.3 | 0.3 | 0.4 | 0.3 | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 0.3 | 0.5 |
| 18:2(n-6) | 0.5 | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.6 | 0.7 | 0.5 | 1.2 | 0.6 | 1.2 |
| 18:3(n-6) | 0.2 | 0.4 | 0.3 | 0.6 | 0.3 | 0.3 | 0.4 | 0.4 | 0.3 | 0.6 | 0.3 | 0.7 |
| 18:3(n-3) | 0.2 | 0.2 | 0.5 | 0.3 | 0.1 | 0.2 | 0.2 | 0.3 | 0.2 | 0.8 | 0.5 | 0.8 |
| 18:4(n-3) | 2.0 | 2.3 | 2.3 | 2.1 | 2.0 | 2.0 | 2.1 | 3.1 | 2.3 | 5.7 | 2.8 | 4.4 |
| 20:1(n-11) | nd | nd | tr | tr | tr | tr | tr | 0.1 | 0.1 | 0.2 | tr | 0.2 |
| 20:4(n-3) | 0.3 | 0.4 | 0.3 | 0.3 | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 | 0.7 | 0.4 | 0.7 |
| 20:5(n-3) | 23.6 | 25.0 | 28.0 | 22.1 | 31.9 | 28.7 | 26.7 | 23.2 | 28.6 | 14.1 | 20.4 | 11.0 |
| ΣOBFA | 1.5 | 0.8 | 1.0 | 1.1 | 1.9 | 1.1 | 1.3 | 1.6 | 1.2 | 1.3 | 1.2 | 1.6 |
| ΣSFA | 17.6 | 14.7 | 14.3 | 15.0 | 15.7 | 16.0 | 15.0 | 16.8 | 15.7 | 16.5 | 16.5 | 19.0 |
| ΣMUFA | 41.0 | 41.4 | 37.5 | 40.7 | 35.0 | 39.6 | 40.8 | 42.5 | 35.9 | 49.5 | 41.1 | 48.8 |
| ΣPUFA | 41.4 | 44.0 | 48.1 | 44.3 | 49.3 | 44.4 | 44.2 | 40.7 | 48.5 | 33.9 | 42.4 | 32.2 |
| DHA/EPA | 0.08 | 0.19 | 0.17 | 0.29 | 0.14 | 0.09 | 0.15 | 0.11 | 0.17 | 0.35 | 0.37 | 0.57 |
| (n-3)/(n-6) | 24.5 | 25.8 | 31.1 | 19.6 | 29.7 | 25.2 | 22.8 | 19.3 | 27.9 | 12.1 | 25.4 | 9.7 |
| TL[a] | 493.0 | 618.6 | 645.9 | 694.8 | 443.5 | 707.2 | 702.2 | 638.7 | 565.9 | 548.3 | 735.3 | 652.8 |
| WE | 83.0 | 81.5 | 82.8 | 83.3 | 82.8 | 75.7 | 86.5 | 87.9 | 88.7 | 90.0 | 80.1 | 91.7 |
| UC | 0.47 | 0.40 | 0.47 | 0.39 | 0.47 | 0.47 | 0.42 | 0.36 | 0.43 | 0.26 | 0.39 | 0.25 |

[a]Total lipid: sum of all lipid classes (μg copepod$^{-1}$)

Table 5.6. Lipid variables with significant PCA loadings in deep-layer *Metridia longa* CVI (female) collected in the North Water during autumn 1999.

| Lipid (%) | 01 | 02 | 03 | 06 | 14 | 32 | 35 | 40 | 45 | *54a | *54b | *58 | *66 | *76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:1 | 0.2 | 0.1 | 0.1 | 0.2 | 0.1 | 0.5 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | nd | 2.1 | 0.9 |
| 15:0 | 0.2 | 0.2 | 0.2 | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | 0.2 | 0.2 | 0.2 | 1.1 | 0.1 | 0.2 |
| 15:1 | 0.4 | 0.4 | 0.3 | 0.2 | 0.2 | 0.3 | 0.3 | 0.2 | 0.3 | 0.3 | 0.3 | 1.0 | tr | 0.1 |
| 16:0 | 6.0 | 5.6 | 5.5 | 4.7 | 4.8 | 5.2 | 6.0 | 4.8 | 6.2 | 6.3 | 5.6 | 13.1 | 4.6 | 6.6 |
| 16:1(n-7) | 30.6 | 31.6 | 32.3 | 23.5 | 22.7 | 22.2 | 35.4 | 31.0 | 31.1 | 28.8 | 27.7 | 19.1 | 16.0 | 14.0 |
| 16:1(n-5) | 0.3 | 0.4 | 0.3 | 0.2 | 0.2 | 0.2 | nd | 0.2 | 0.3 | 0.3 | 0.3 | 0.8 | 0.1 | 0.2 |
| 16:3(n-4) | 0.6 | 0.9 | 0.9 | 0.7 | 0.7 | 0.5 | 0.6 | 0.7 | 0.7 | 0.6 | 0.6 | 0.8 | 0.1 | 0.3 |
| 16:4(n-1) | 1.1 | 1.0 | 0.9 | 0.9 | 0.9 | 0.9 | 1.0 | 0.9 | 1.2 | 0.6 | 1.0 | 0.3 | 0.3 | 0.8 |
| 18:1(n-7) | 2.6 | 2.4 | 2.5 | 1.8 | 1.8 | 2.0 | 2.4 | 1.7 | 2.4 | 2.1 | 2.2 | 3.2 | 1.4 | 1.7 |
| 18:1(n-5) | 0.5 | 0.4 | 0.4 | 0.3 | 0.4 | 0.3 | 0.4 | 0.3 | 0.5 | 0.6 | 0.5 | 1.3 | 1.1 | 0.7 |
| 18:2(n-6) | 1.3 | 1.3 | 1.2 | 1.1 | 1.1 | 1.2 | 1.3 | 1.4 | 1.0 | 1.7 | 1.4 | 2.2 | 4.9 | 2.3 |
| 18:2(n-4) | 0.3 | 0.2 | 0.2 | nd | 0.1 | 0.1 | 0.2 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 | nd | nd |
| 18:3(n-3) | 0.6 | 0.7 | 0.6 | 0.6 | 0.7 | 0.3 | 0.4 | 0.4 | 0.4 | 0.5 | 0.5 | 0.5 | 0.8 | 1.0 |
| 20:4(n-3) | 0.4 | 0.5 | 0.4 | 0.4 | 0.4 | 0.9 | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 | nd | 0.4 | 0.5 |
| 22:1(n-9) | 0.2 | 0.6 | 0.3 | 0.3 | 0.2 | 0.3 | 0.6 | 0.4 | 0.6 | 0.5 | 0.6 | 0.4 | nd | 0.2 |
| 22:5(n-3) | 0.5 | 0.6 | 0.5 | 2.8 | 0.9 | 1.8 | 0.4 | nd | 0.5 | 0.9 | 0.7 | 0.2 | 4.4 | 1.6 |
| 22:6(n-3) | 3.7 | 4.6 | 6.5 | 14.1 | 17.4 | 13.4 | 3.5 | 4.6 | 4.4 | 8.4 | 9.8 | 9.8 | 22.2 | 16.1 |
| 24:1 | 0.8 | 1.2 | 0.9 | 1.9 | 3.9 | 1.9 | 1.1 | 1.0 | 0.7 | 0.8 | 0.6 | 1.9 | 4.3 | 8.5 |
| ΣOBFA | 1.7 | 1.2 | 1.2 | 0.6 | 0.9 | 1.3 | 1.4 | 0.8 | 1.0 | 1.2 | 1.0 | 3.9 | 1.1 | 0.5 |
| ΣSFA | 9.9 | 8.8 | 8.7 | 7.7 | 8.1 | 8.5 | 9.5 | 7.6 | 10.5 | 10.4 | 8.9 | 20.2 | 8.0 | 11.0 |
| ΣMUFA | 58.1 | 57.7 | 56.8 | 46.5 | 45.6 | 46.2 | 62.1 | 59.1 | 54.6 | 53.8 | 49.2 | 50.8 | 40.0 | 49.2 |
| 16:1/16:0 | 5.1 | 5.6 | 5.9 | 5.0 | 4.8 | 4.3 | 5.9 | 6.4 | 5.0 | 4.6 | 5.0 | 1.5 | 3.5 | 2.1 |
| DHA/EPA | 0.23 | 0.28 | 0.39 | 0.78 | 1.06 | 0.72 | 0.25 | 0.28 | 0.28 | 0.57 | 0.50 | 0.88 | 1.61 | 1.99 |
| TFA | 86.9 | 96.4 | 92.5 | 137.0 | 127.8 | 167.6 | 96.7 | 51.0 | 61.1 | 35.1 | 67.0 | 16.0 | 128.6 | 35.4 |
| TL | 152.7 | 147.1 | 144.6 | 186.6 | 199.8 | 213.0 | 134.4 | 143.5 | 163.9 | 82.1 | 186.4 | 40.3 | 156.2 | 118.3 |
| WE | 68.4 | 67.0 | 74.3 | 72.6 | 69.2 | 71.4 | 77.4 | 79.3 | 77.4 | 75.9 | 69.9 | 49.3 | 75.4 | 71.5 |
| TG | 14.6 | 15.9 | 11.8 | 10.3 | 11.3 | 10.5 | 12.6 | 13.6 | 15.1 | 5.9 | 14.8 | 4.6 | 3.7 | 6.2 |
| PL | 11.9 | 11.9 | 9.2 | 9.1 | 11.3 | 11.3 | 4.7 | 3.9 | 3.2 | 13.9 | 12.0 | 37.7 | 15.9 | 16.2 |
| WE/DM[a] | 229.2 | 197.5 | 265.9 | 267.7 | 302.1 | 287.4 | 219.6 | 276.1 | 241.0 | 137.1 | 264.7 | 54.5 | 257.6 | 189.6 |
| % LIPID | 33.5 | 29.5 | 35.7 | 36.9 | 43.6 | 40.3 | 28.4 | 34.8 | 31.2 | 20.6 | 37.9 | 11.1 | 34.2 | 26.5 |
| UC | 0.26 | 0.25 | 0.21 | 0.26 | 0.26 | 0.30 | 0.20 | 0.22 | 0.24 | 0.21 | 0.32 | 0.03 | 0.31 | 0.27 |

[a]units=μg $mg^{-1}$ (DM=dry mass); TG=triacylglycerol; PL=phospholipid

Table 5.7. Summary of feeding strategies and diets of copepods collected in the surface layer as deduced by Principal Components Analysis (numbers refer to stations).

| | *Calanus hyperboreus* | *Calanus glacialis* |
|---|---|---|
| Herbivory (Diatoms) | 01, 03, 14, 32, 35, 44, 45, 54b, 68 | 01[b], 03[b], 06[b], 32[b], 35[b], 40[b], 44[b], 45[b], 54b[b], 66[b], 68[b] |
| Herbivory (Dinoflagellates) | 06, 40, 66 | 14 |
| Omnivory | 50, 54a, 58, | 54a[b], 76[b] |

Underlined numbers=SE stations
b=bacterial input

Table 5.8. Summary of feeding strategies and diets of copepods collected in the deep layer as deduced by Principal Components Analysis (numbers refer to stations).

| | *Calanus hyperboreus* | *Calanus glacialis* | *Metridia longa* |
|---|---|---|---|
| Herbivory (Diatoms) | 02, 03, 06, 14, 32, 35[d], 45, 54a, 54b[b], 66[d] | 02[b], 03[d], 06[d], 14[d], 32[b], 35[d], 45, 54a, 54b, 66[d] | 01, 02, 03, 06, 14, 32, 35, 40, 45, 54a, 54b |
| Omnivory/ Dinoflagellate-eating | 58[b], 76[b] | 58, 76 | 58[b], 66, 76 |

Underlined numbers=SE stations
b=bacterial input
d=diapause

Table 5.9. Relative fatty acid composition and lipid content of seston collected at 1% light and at depth during autumn in the North Water (error bars represent 1 standard deviation).

| Lipid[b] (%) | NORTHWEST STATIONS (01.02.03.06.14.32.35.40.45) | | SOUTHEAST STATIONS (50.54a.54b.58.66.68.76) | |
|---|---|---|---|---|
| | 1% light[a] | Deep[a] | 1% light[a] | Deep[a] |
| 14:0 | 11.7 ± 1.1 | 10.8 ± 2.2 | 9.7 ± 2.0 | 11.7 ± 5.9 |
| i-15:0 | 0.8 ± 0.3 | 1.1 ± 0.7 | 1.0 ± 0.2 | 2.1 ± 0.9 |
| ai-15:0 | 0.6 ± 0.3 | 1.8 ± 3.8 | 0.8 ± 0.2 | 0.9 ± 0.7 |
| 15:0 | 0.6 ± 0.2 | 0.9 ± 0.3 | 0.6 ± 0.1 | 1.4 ± 1.1 |
| 15:1 | 0.3 ± 0.2 | 0.6 ± 0.4 | 0.3 ± 0.2 | 0.7 ± 0.7 |
| i-16:0 | 0.4 ± 0.4 | 0.4 ± 0.2 | 0.7 ± 0.3 | 0.6 ± 0.5 |
| ai-16:0 | 0.5 ± 0.4 | 0.3 ± 0.2 | 0.7 ± 0.4 | nd |
| 16:0 | 13.5 ± 1.9 | 16.6 ± 2.9 | 14.4 ± 5.0 | 19.5 ± 9.5 |
| 16:1(n-9) | 1.1 ± 0.7 | 0.3 ± 0.3 | 1.4 ± 0.7 | 0.1 ± 0.2 |
| 16:1(n-7) | 24.8 ± 5.3 | 25.2 ± 5.5 | 15.5 ± 4.5 | 17.9 ± 10.1 |
| 16:1(n-5) | 0.7 ± 0.2 | 1.6 ± 2.7 | 0.8 ± 0.2 | 0.5 ± 0.3 |
| i-17:0 | 0.8 ± 0.2 | 0.5 ± 0.4 | 0.9 ± 0.1 | 1.1 ± 1.0 |
| ai-17:0 | 0.4 ± 0.1 | 0.3 ± 0.2 | 0.6 ± 0.3 | 0.2 ± 0.3 |
| 16:2(n-4) | 1.5 ± 0.3 | 0.9 ± 0.3 | 1.6 ± 0.2 | 1.3 ± 0.9 |
| 17:0 | 0.1 ± 0.1 | 0.4 ± 0.2 | 0.4 ± 0.4 | 0.5 ± 0.4 |
| 16:3(n-4) | 1.1 ± 0.4 | 0.7 ± 0.3 | 2.3 ± 0.6 | 1.3 ± 0.9 |
| 16:4(n-1) | 3.7 ± 1.1 | 1.4 ± 0.6 | 2.8 ± 0.4 | 1.5 ± 0.9 |
| 18:0 | 1.0 ± 0.2 | 4.1 ± 2.1 | 1.9 ± 0.7 | 5.0 ± 2.7 |
| 18:1(n-9) | 3.5 ± 1.7 | 7.2 ± 4.4 | 5.1 ± 2.6 | 7.1 ± 5.7 |
| 18:1(n-7) | 1.5 ± 0.5 | 1.3 ± 0.6 | 1.8 ± 0.5 | 1.2 ± 0.7 |
| 18:2(n-6) | 1.0 ± 0.3 | 2.1 ± 1.3 | 1.7 ± 0.7 | 1.5 ± 0.9 |
| 18:2(n-4) | 0.5 ± 0.4 | 1.3 ± 0.8 | 0.8 ± 0.6 | 2.3 ± 1.6 |
| 18:4(n-3) | 1.6 ± 0.4 | 1.3 ± 0.4 | 3.1 ± 1.3 | 1.4 ± 1.2 |
| 20:1(n-11) | 0.5 ± 0.2 | 0.4 ± 0.3 | 2.2 ± 1.3 | 0.7 ± 0.8 |
| 20:1(n-9) | 0.4 ± 0.3 | 1.1 ± 1.1 | 0.6 ± 0.3 | 1.6 ± 1.3 |
| 20:4(n-6) | 0.4 ± 0.1 | 0.1 ± 0.1 | 0.4 ± 0.3 | 0.1 ± 0.2 |
| 20:5(n-3) | 16.9 ± 1.9 | 7.9 ± 3.0 | 12.7 ± 2.4 | 7.7 ± 3.8 |
| 22:6(n-3) | 5.0 ± 1.5 | 2.5 ± 1.4 | 8.5 ± 3.6 | 3.7 ± 2.2 |
| | | | | |
| ΣOBFA | 4.6 ± 1.3 | 6.4 ± 4.1 | 6.1 ± 1.0 | 7.8 ± 3.7 |
| ΣSFA | 30.4 ± 2.1 | 37.5 ± 4.8 | 32.0 ± 5.6 | 43.5 ± 20.4 |
| ΣMUFA | 34.9 ± 3.8 | 40.6 ± 5.3 | 29.6 ± 3.3 | 32.1 ± 17.2 |
| ΣPUFA | 34.7 ± 4.6 | 21.8 ± 5.5 | 38.4 ± 3.9 | 24.4 ± 5.9 |
| 16:1(n-7)/16:0 | 1.8 ± 0.3 | 1.5 ± 0.3 | 1.2 ± 0.4 | 1.3 ± 1.0 |
| $C_{16}/C_{18}$ | 5.9 ± 1.8 | 3.5 ± 1.0 | 3.2 ± 1.5 | 3.7 ± 2.4 |
| $C_{16}$PUFA/$C_{18}$PUFA | 2.1 ± 0.7 | 0.8 ± 0.3 | 1.2 ± 0.4 | 0.9 ± 0.6 |
| $C_{18}$PUFA+$C_{22}$PUFA | 9.5 ± 2.6 | 8.7 ± 2.4 | 16.3 ± 4.8 | 10.4 ± 3.4 |
| DHA/EPA | 0.30 ± 0.09 | 0.31 ± 0.13 | 0.72 ± 0.41 | 0.51 ± 0.17 |
| (n-3)/(n-6) | 15.5 ± 3.2 | 7.9 ± 6.5 | 12.5 ± 3.6 | 5.9 ± 4.2 |
| | | | | |
| TL (ng $ml^{-1}$) | 49.9 ± 16.8 | 11.6 ± 6.5 | 40.3 ± 12.1 | 10.3 ± 6.8 |
| TFA (ng $ml^{-1}$) | 30.7 ± 13.0 | 5.8 ± 3.8 | 19.1 ± 5.9 | 3.8 ± 2.4 |
| PUFA (ng $ml^{-1}$) | 10.5 ± 3.9 | 1.1 ± 0.6 | 7.4 ± 2.0 | 0.8 ± 0.6 |

[a]sampling depth as in last column of Table 5.1 (actual 1% light depth as in column 3)
[b]data not shown for fatty acids ≤1% of the total in all samples (exceptions are odd/and or branched acids and 20:4(n-6); see Appendices 13-14)
nd=not detected

Table 5.10. Concentrations of various seston parameters measured at 1% light during autumn in the North Water (error bars are 1 standard deviation).

| Seston measurement | NORTHWEST | SOUTHEAST |
|---|---|---|
| POC[a] ($\mu g\ l^{-1}$) | 276.0 ± 125.2 | 160.5 ± 86.1 |
| C:Chl[a] | 34.0 ± 22.6 | 43.3 ± 29.3 |
| 1° prod., total[a] (mg C $m^{-3}\ d^{-1}$) | 16.3 ± 33.5 | 1.5 ± 1.0 |
| 1° prod., > 5μm[a] (mg C $m^{-3}\ d^{-1}$) | 10.0 ± 20.0 | 0.8 ± 0.4 |
| 1° prod., < 5μm[a] (mg C $m^{-3}\ d^{-1}$) | 6.3 ± 13.6 | 0.7 ± 0.6 |
| Total autotrophs[b] ($\mu g\ C\ l^{-1}$) | 101.3 ± 48.1 | 86.2 ± 34.8 |
| *Chaetoceros socialis*[b] ($\mu g\ C\ l^{-1}$) | 71.8 ± 39.9 | 47.8 ± 27.8 |
| Auto. Dinoflagellates[b] ($\mu g\ C\ l^{-1}$) | 2.1 ± 2.4 | 6.7 ± 3.5 |
| Bacterial biomass[c] (mg C $m^{-2}$) | 76.3 ± 48.7 | 118.6 ± 49.0 |

[a]data provided by B. Klein; [b]data from Booth et al. (2002); [c]data provided by R. Rivkin; POC=particulate organic carbon; C:Chl=carbon:chlorophyll; prod=production; auto=autotrophic

## 5.10 Figures

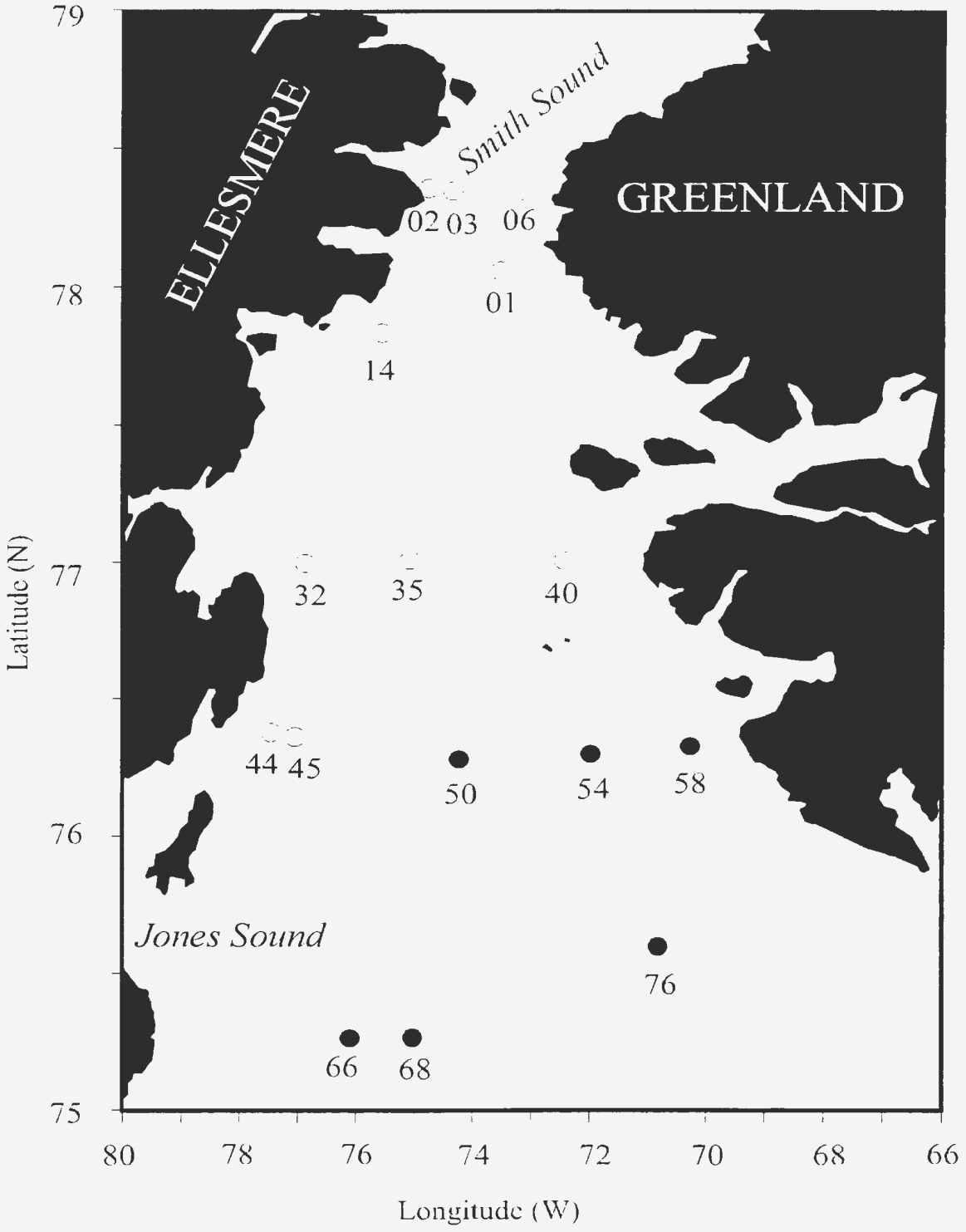



Figure 5.1. Stations sampled in the North Water during autumn 1999 (filled circles are southeastern stations).

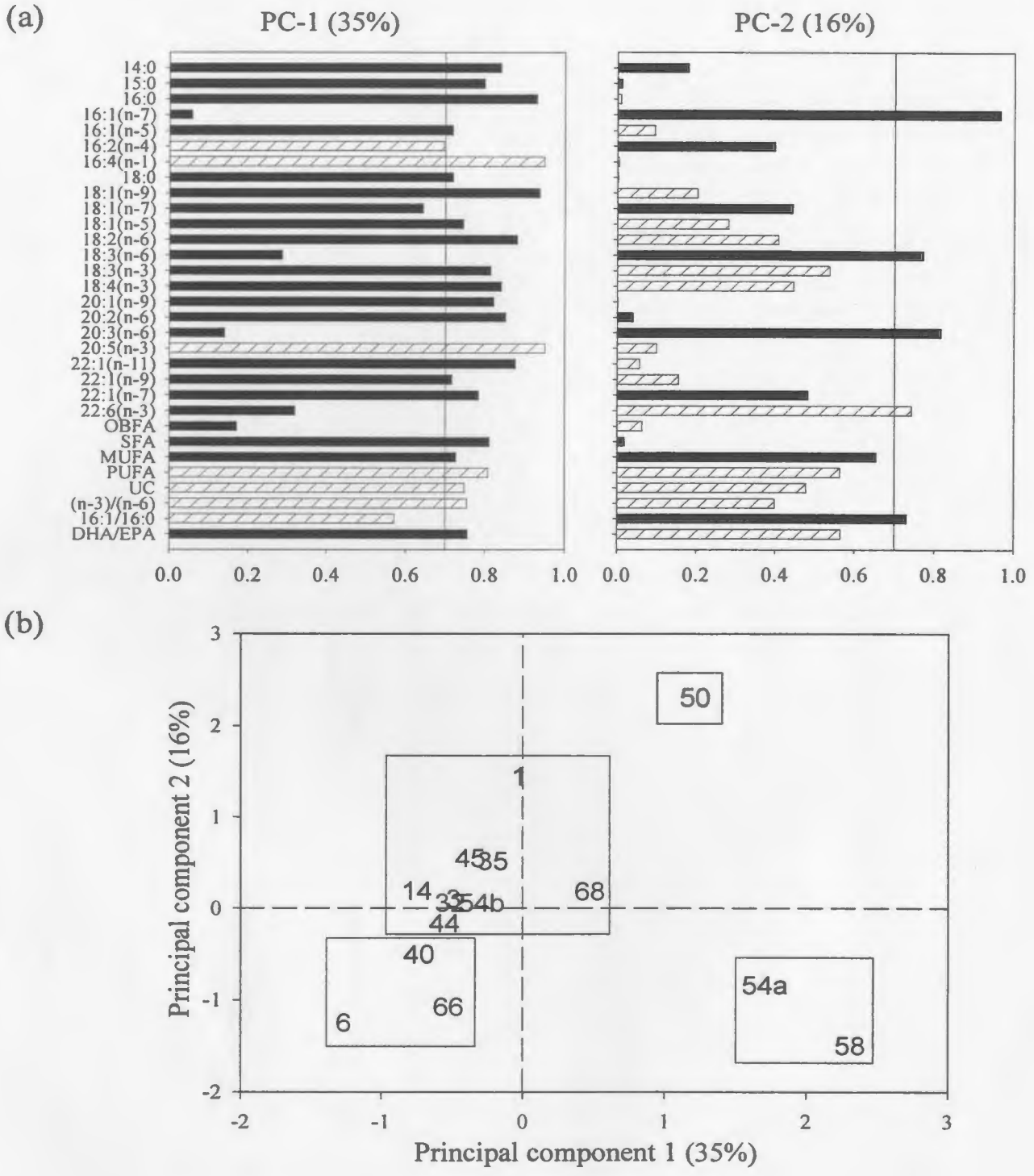



Figure 5.2. Results of PCA for surface-layer samples of *Calanus hyperboreus* CV: (a) loadings of variables on principal components 1 and 2 (solid bars=positive loadings; hatched bars=negative loadings), (b) positions of station scores on principal component axes; boxes around station numbers represent results of hierarchical cluster analysis.

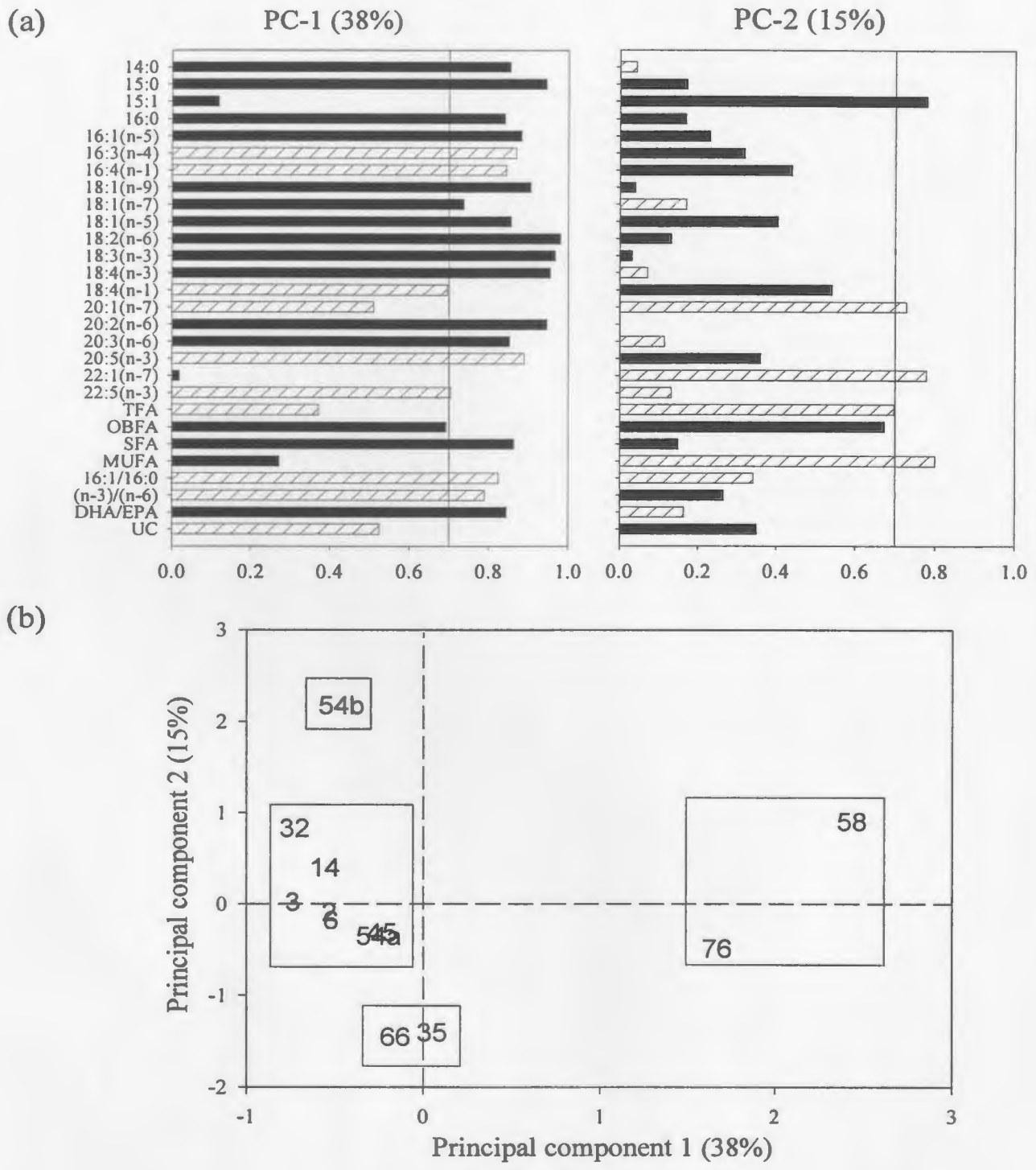



Figure 5.3. Results of PCA for deep-layer samples of *Calanus hyperboreus* CV: (a) loadings of variables on principal components 1 and 2 (solid bars=positive loadings; hatched bars=negative loadings), (b) positions of station scores on principal component axes.

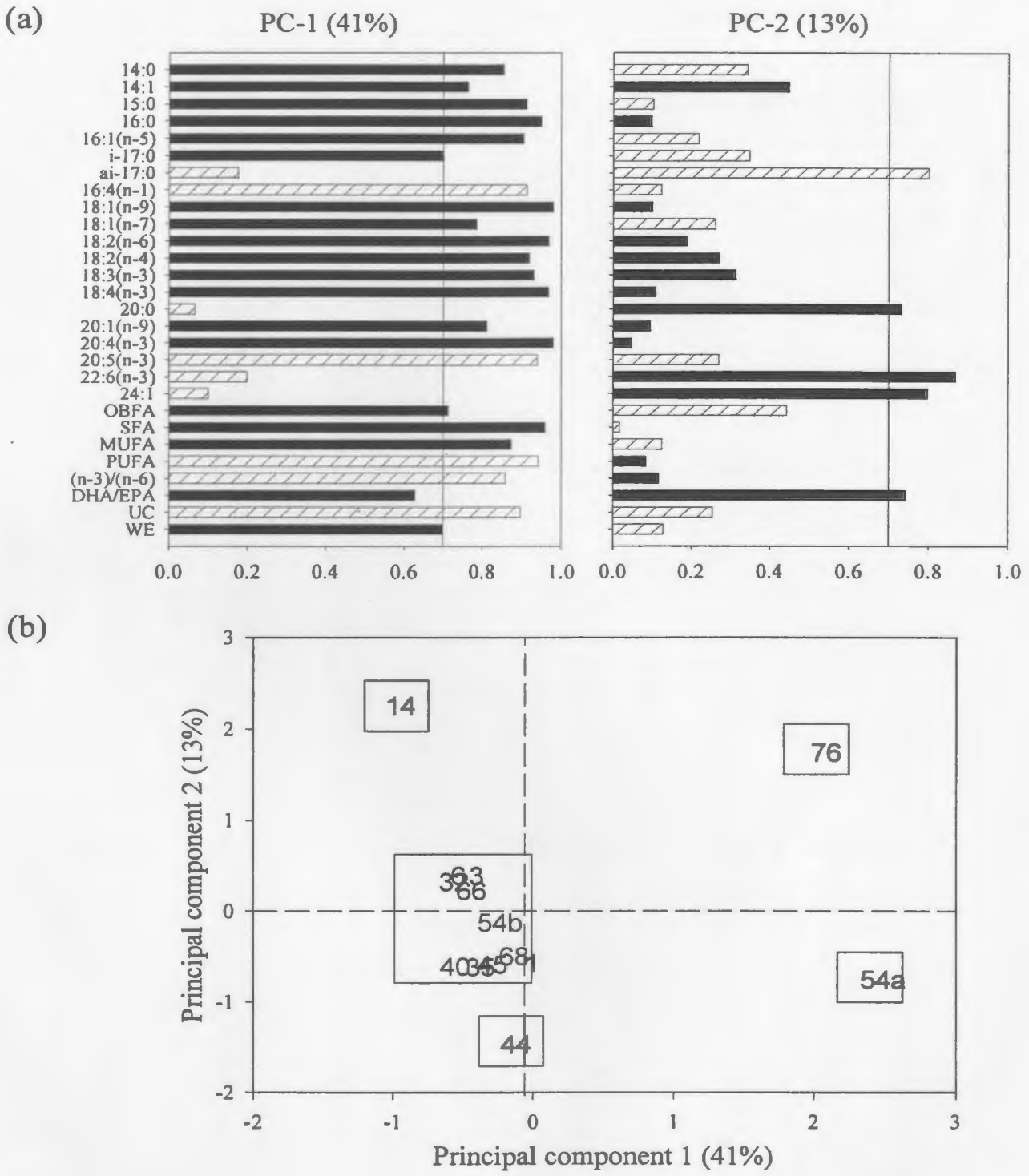



Figure 5.4. Results of PCA for surface-layer samples of *Calanus glacialis* CV: (a) loadings of variables on principal components 1 and 2 (solid bars=positive loadings; hatched bars=negative loadings), (b) positions of station scores on principal component axes.

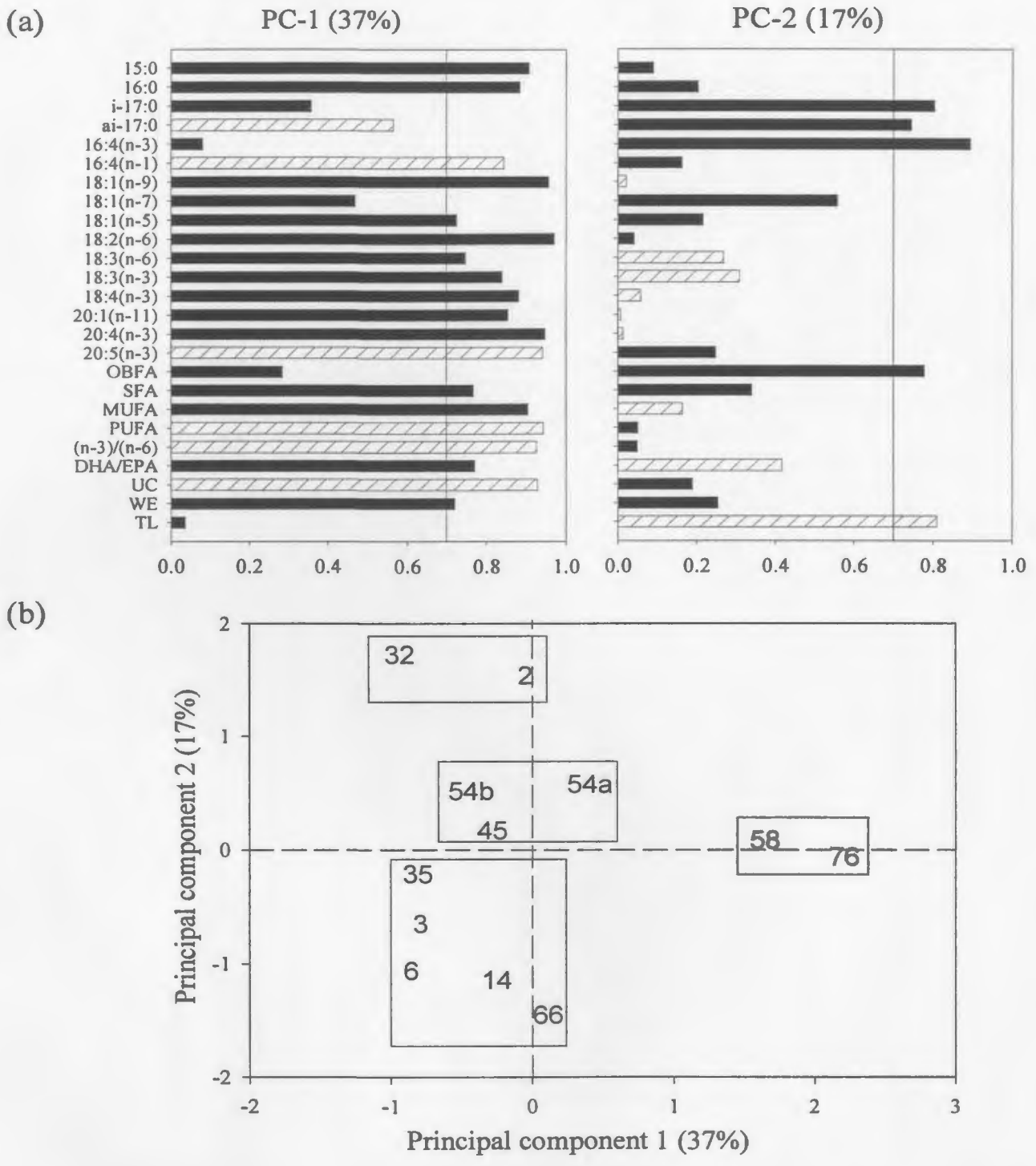



Figure 5.5. Results of PCA for deep-layer samples of *Calanus glacialis* CV: (a) loadings of variables on principal components 1 and 2 (solid bars=positive loadings; hatched bars=negative loadings), (b) positions of station scores on principal component axes.

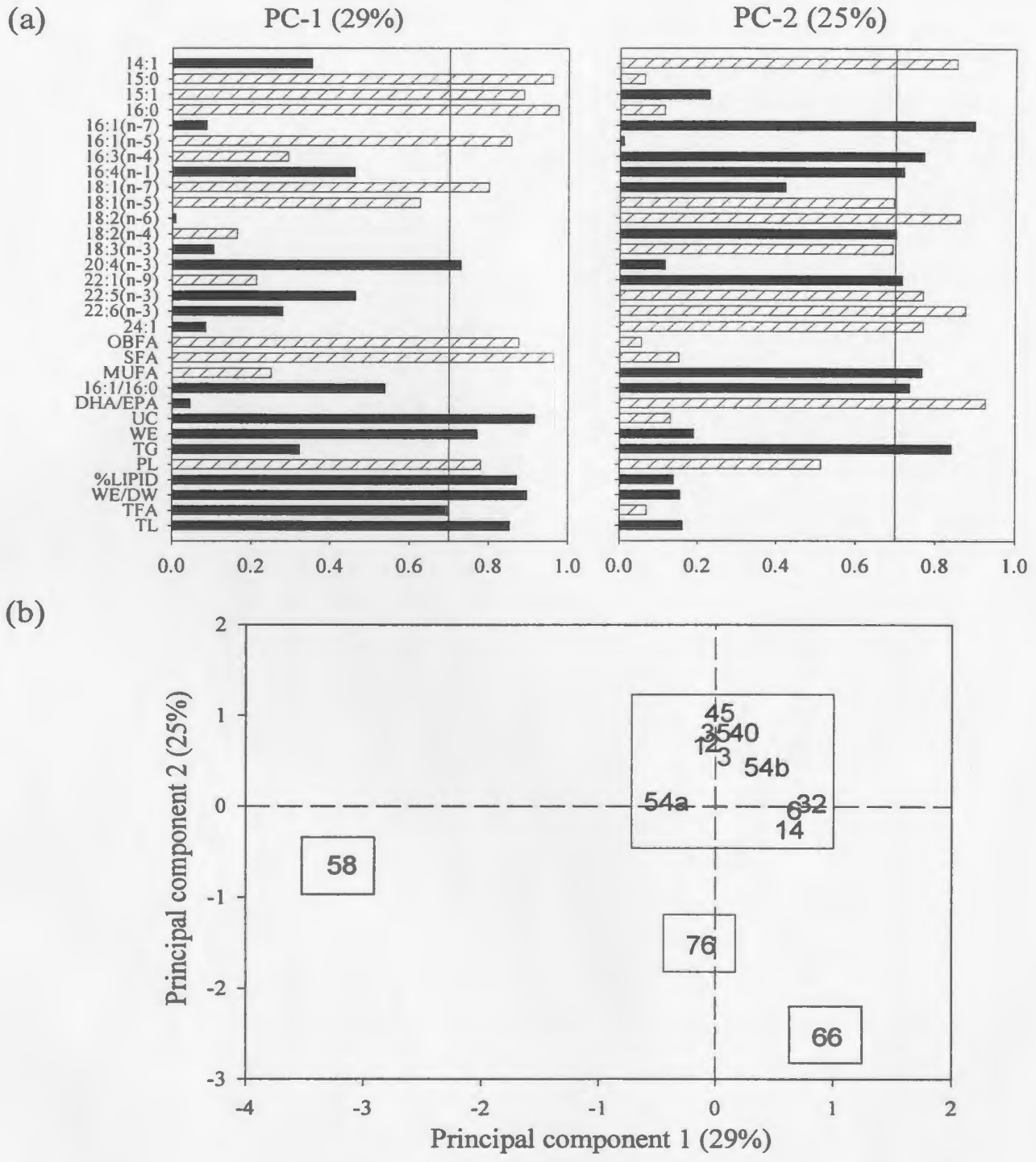



Figure 5.6. Results of PCA for deep-layer samples of *Metridia longa* CVI-F: (a) loadings of variables on principal components 1 and 2 (solid bars=positive loadings; hatched bars=negative loadings), (b) positions of station scores on principal component axes.

## Chapter 6. Thesis summary

This thesis reports the first comprehensive description of copepod and seston lipids in the North Water, the largest and most productive polynya in the Canadian Arctic. A new wax-ester based omnivory index, the unsaturation coefficient (UC), was developed and tested. The utility of bacterial fatty acids (odd and/or branched; 18:1(n-7)) as omnivory indices for pelagic copepods was also investigated. Using these tools, in addition to extensive fatty acid and lipid class data, species-specific and spatial patterns in copepod omnivory were investigated throughout the polynya. This work focused on omnivory by three numerically dominant copepods: *Calanus hyperboreus* CV, *C. glacialis* CV and *Metridia longa* CVI (females).

The lipid data revealed that all species ingested non-phytoplankton prey, but to varying degrees. Levels of several omnivory indices demonstrated that *Metridia longa* was the most omnivorous of the three species studied. However, bacterial fatty acid patterns in *Calanus glacialis* suggest that this species is most closely linked to the microbial food web. It is probable that the omnivorous feeding habit of each species has evolved to exploit particular prey in the Arctic Ocean and that these three copepods represent a trophic continuum under the conditions encountered in this study. *M. longa* may be more likely to eat small metazoans and copepod eggs and *C. glacialis*, marine snow and dying phytoplankton cells. *C. hyperboreus* is probably more herbivorous. Principal components analysis revealed that all copepods fed omnivorously in the

southeastern corner of the polynya where the microbial loop was active. Elsewhere, phytoplankton was more abundant and the copepods fed herbivorously.

Copepods in the North Water responded quickly to transient bloom events and dietary shifts were mirrored in their lipid signatures. These changes imply a certain degree of adaptability on the part of the copepods. Arctic calanoids are thus not strict re-packagers of photosynthetically produced carbon and probably have a highly varied diet. Because of the possible stabilizing role of omnivory in food webs, such small-scale processes should be considered when including zooplankton in dynamic ecosystem models.

The heterotrophic markers explored in this thesis were very useful at demonstrating connections between pelagic copepods and the microbial food web. Their sensitivity on both species-specific and spatial scales suggests much promise for their use in other pelagic ecosystems, especially those characterized by oligotrophy. A continued interest in the assessment of omnivory may lead to the development of quantitative, true *in situ* methods. To this end, future studies should focus on the lability and *in vivo* turnover of microbial markers. Detailed information on microplankton assemblages and biochemical signatures of heterotrophic prey in systems of interest are also required. Studies that combine several methods to deduce diet and feeding strategy (e.g., lipid

biomarkers, pigments, incubations, multivariate statistics, stable isotopes, gut analysis, faecal pellet analysis, modeling) will probably be fruitful.

# Appendices

Appendix 1. Complete lipid class composition of surface-layer *Calanus hyperboreus* CV sampled in the North Water Polynya during fall 1999 (column headings refer to stations; HC=hydrocarbons, WE=wax esters, ME=methyl esters, KET=ketones, TG=triacylglycerols, FFA=free fatty acids, ALC=alcohols, ST=sterols, DG=diacylglycerols, AMPL=acetone-mobile polar lipids, PL=phospholipids, TL=total lipid, DM=dry mass).

| Lipid Class (%) | 01 | 03 | 06 | 14-A | 14-B | 14-C | 32 | 35 | 40 | 44 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HC | 3.12 | 3.61 | 4.04 | 2.80 | 2.73 | 2.27 | 4.12 | 2.62 | 2.30 | 3.61 | 5.87 |
| WE | 77.80 | 87.04 | 89.16 | 91.46 | 92.55 | 91.62 | 88.63 | 87.27 | 83.75 | 86.32 | 83.02 |
| ME | 1.18 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 11.49 | 3.06 | 1.81 | 0.00 | 0.80 | 0.16 | 1.96 | 2.02 | 2.72 | 3.02 | 1.90 |
| FFA | 0.25 | 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.22 | 0.00 |
| ALC | 0.65 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ST | 0.44 | 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.61 | 0.00 | 0.00 | 0.00 | 0.00 |
| DG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| AMPL | 1.95 | 2.17 | 1.65 | 3.22 | 2.58 | 3.22 | 0.74 | 4.66 | 7.42 | 2.28 | 3.64 |
| PL | 3.13 | 3.71 | 3.34 | 2.52 | 1.35 | 2.72 | 3.94 | 3.43 | 3.81 | 4.54 | 5.57 |
| | | | | | | | | | | | |
| WE (µg) | 1347.1 | 913.0 | 1017.9 | 805.6 | 1240.3 | 959.1 | 1183.3 | 860.5 | 1087.4 | 803.1 | 1068.3 |
| TL (µg) | 1731.5 | 1048.9 | 1141.7 | 880.9 | 1340.1 | 1046.8 | 1335.0 | 986.1 | 1298.4 | 930.4 | 1286.9 |
| DM (mg) | 1.72 | -- | 1.88 | 1.59 | 1.59 | 1.59 | 1.63 | 2.41 | 2.52 | 1.76 | -- |

Appendix 1. Continued.

| Lipid Class (%) | 50 | 54a | 58 | 66 | 68 |
|---|---|---|---|---|---|
| HC | 3.98 | 1.69 | 3.56 | 2.74 | 2.82 |
| WE | 89.75 | 91.49 | 83.77 | 82.97 | 88.79 |
| ME | 0.00 | 0.00 | 0.00 | 0.58 | 0.00 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 1.77 | 0.00 | 4.17 | 1.20 | 1.32 |
| FFA | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ALC | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ST | 0.40 | 0.00 | 0.00 | 2.00 | 0.22 |
| DG | 0.00 | 0.00 | 0.00 | 0.32 | 2.79 |
| AMPL | 1.73 | 2.89 | 1.46 | 4.29 | 1.85 |
| PL | 2.37 | 3.93 | 7.04 | 5.88 | 2.21 |
| | | | | | |
| WE (µg) | 1103.4 | 848.1 | 731.2 | 1049.9 | 1414.1 |
| TL (µg) | 1229.5 | 927.0 | 872.9 | 1265.4 | 1592.7 |
| DM (mg) | 2.51 | 1.45 | 2.87 | 2.09 | 2.33 |

Appendix 2. Complete lipid class composition of deep-layer *Calanus hyperboreus* CV (NOW: fall 1999).

| Lipid Class (%) | 02-A | 02-B | 02-C | 03 | 06 | 14 | 32 | 35 | 45 | 54a | 54b |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HC | 2.47 | 3.05 | 2.60 | 2.93 | 3.16 | 2.78 | 2.14 | 1.52 | 1.51 | 1.73 | 3.70 |
| WE | 82.35 | 83.76 | 83.03 | 85.02 | 89.45 | 81.76 | 87.12 | 90.19 | 92.45 | 90.98 | 88.06 |
| ME | 0.00 | 0.00 | 0.00 | 0.11 | 0.00 | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 9.89 | 7.22 | 6.52 | 3.32 | 3.28 | 2.59 | 2.01 | 4.82 | 1.79 | 3.15 | 3.15 |
| FFA | 0.00 | 0.00 | 0.00 | 0.00 | 0.29 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.48 |
| ALC | 0.00 | 0.00 | 0.00 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.37 |
| ST | 0.30 | 0.00 | 0.35 | 0.63 | 0.00 | 1.23 | 1.44 | 0.00 | 0.84 | 0.00 | 0.46 |
| DG | 0.00 | 0.00 | 0.00 | 0.00 | 0.39 | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| AMPL | 0.59 | 1.73 | 0.92 | 3.98 | 0.75 | 5.87 | 3.51 | 1.39 | 1.74 | 1.21 | 1.43 |
| PL | 4.40 | 4.24 | 6.58 | 3.93 | 2.68 | 5.06 | 3.78 | 2.08 | 1.68 | 2.93 | 2.35 |
| WE (µg) | 1302.7 | 989.9 | 1197.6 | 1330.2 | 1829.8 | 1134.6 | 1613.1 | 1850.7 | 1364.2 | 1342.2 | 1645.4 |
| TL (µg) | 1581.9 | 1181.8 | 1442.4 | 1567.7 | 2045.8 | 1387.7 | 1845.7 | 2051.9 | 1475.6 | 1475.3 | 1868.4 |
| DM (mg) | 2.25 | 2.25 | 2.25 | 3.13 | 3.71 | 3.51 | 3.27 | 2.97 | 2.18 | 1.20 | 3.56 |

Appendix 2. Continued.

| Lipid Class (%) | 58 | 66 | 76-A | 76-B | 76-C |
|---|---|---|---|---|---|
| HC | 1.42 | 2.18 | 1.06 | 2.02 | 1.04 |
| WE | 93.13 | 85.52 | 92.22 | 90.98 | 91.96 |
| ME | 0.00 | 0.51 | 0.00 | 0.00 | 0.00 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 0.38 | 2.76 | 0.00 | 1.66 | 0.72 |
| FFA | 0.00 | 0.00 | 0.00 | 0.71 | 0.90 |
| ALC | 0.00 | 0.60 | 0.00 | 0.00 | 0.31 |
| ST | 0.35 | 0.96 | 0.82 | 0.00 | 0.19 |
| DG | 0.00 | 0.42 | 0.00 | 0.00 | 0.00 |
| AMPL | 2.20 | 2.24 | 1.58 | 1.23 | 1.64 |
| PL | 2.53 | 4.80 | 4.31 | 3.40 | 3.25 |
| WE (µg) | 1379.3 | 1806.7 | 1396.9 | 1737.9 | 1371.7 |
| TL (µg) | 1481.1 | 2112.6 | 1514.7 | 1910.2 | 1491.7 |
| DM (mg) | 3.23 | 3.17 | 3.96 | 3.96 | 3.96 |

Appendix 3. Complete lipid class composition of surface-layer *Calanus glacialis* CV (NOW; fall 1999).

| Lipid Class (%) | 01 | 03 | 06 | 14 | 32 | 35 | 40 | 44 | 45 | 54a |
|---|---|---|---|---|---|---|---|---|---|---|
| HC | 2.02 | 3.10 | 2.88 | 1.30 | 1.76 | 2.79 | 2.02 | 1.59 | 1.12 | 1.94 |
| WE | 84.66 | 81.11 | 83.58 | 75.03 | 76.82 | 84.72 | 84.66 | 76.11 | 82.87 | 90.55 |
| ME | 0.00 | 0.35 | 0.00 | 1.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.85 | 0.00 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 8.26 | 6.52 | 4.95 | 7.90 | 9.73 | 5.00 | 8.26 | 16.29 | 7.72 | 3.10 |
| FFA | 0.00 | 1.10 | 0.00 | 0.00 | 0.72 | 0.00 | 0.00 | 0.00 | 0.91 | 0.00 |
| ALC | 0.00 | 0.03 | 0.00 | 0.11 | 4.51 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ST | 0.00 | 0.50 | 0.42 | 0.82 | 0.38 | 0.00 | 0.00 | 0.00 | 0.53 | 0.00 |
| DG | 0.00 | 0.00 | 0.59 | 0.53 | 0.00 | 0.00 | 0.00 | 0.00 | 0.30 | 0.00 |
| AMPL | 1.92 | 4.30 | 4.46 | 5.71 | 2.93 | 4.39 | 1.92 | 2.31 | 3.07 | 3.25 |
| PL | 3.13 | 3.00 | 3.12 | 7.51 | 3.16 | 3.10 | 3.13 | 3.70 | 2.61 | 1.16 |
| WE (µg) | 398.1 | 424.1 | 340.4 | 400.2 | 344.3 | 338.8 | 465.2 | 337.8 | 372.3 | 315.3 |
| TL (µg) | 521.1 | 522.9 | 407.3 | 533.4 | 448.1 | 399.9 | 549.4 | 443.8 | 449.3 | 348.2 |
| DM (mg) | 0.86 | -- | 1.01 | 1.20 | 1.01 | 0.71 | 1.20 | 0.81 | 1.12 | 0.99 |

Appendix 3. Continued.

| Lipid Class (%) | 54b-A | 54b-B | 54b-C | 66 | 68 | 76 |
|---|---|---|---|---|---|---|
| HC | 1.42 | 1.94 | 1.67 | 1.18 | 1.45 | 0.88 |
| WE | 86.55 | 87.54 | 88.47 | 81.85 | 81.31 | 89.40 |
| ME | 2.31 | 1.33 | 1.60 | 1.65 | 2.40 | 1.22 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 5.49 | 5.24 | 4.99 | 6.82 | 5.53 | 2.31 |
| FFA | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 | 0.00 |
| ALC | 0.00 | 0.00 | 0.00 | 0.00 | 0.22 | 1.26 |
| ST | 0.00 | 0.08 | 0.00 | 1.92 | 2.11 | 0.00 |
| DG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| AMPL | 2.05 | 2.10 | 1.35 | 3.75 | 1.77 | 2.22 |
| PL | 2.18 | 1.78 | 1.91 | 2.77 | 5.21 | 2.71 |
| WE (µg) | 489.8 | 440.9 | 542.3 | 485.7 | 328.9 | 470.4 |
| TL (µg) | 565.9 | 503.79 | 613.0 | 593.4 | 404.5 | 526.2 |
| DM (mg) | 0.84 | 0.84 | 0.84 | 0.72 | 0.91 | -- |

Appendix 4. Complete lipid class composition of deep-layer *Calanus glacialis* CV (NOW; fall 1999).

| Lipid Class (%) | 02-A | 02-B | 02-C | 03 | 06 | 14 | 32-A | 32-B | 32-C | 35 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HC | 1.92 | 2.27 | 2.24 | 1.46 | 1.23 | 1.63 | 1.89 | 1.93 | 1.75 | 1.96 | 1.78 |
| WE | 88.19 | 83.20 | 77.65 | 81.51 | 82.78 | 83.25 | 81.47 | 83.30 | 83.68 | 75.70 | 86.48 |
| ME | 0.00 | 0.00 | 0.00 | 0.94 | 2.39 | 0.98 | 2.12 | 1.17 | 1.85 | 0.00 | 0.83 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 3.35 | 8.42 | 13.50 | 8.13 | 7.87 | 8.60 | 7.52 | 8.11 | 6.12 | 16.67 | 7.04 |
| FFA | 0.21 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.00 | 0.00 | 0.00 |
| ALC | 0.00 | 0.00 | 1.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ST | 0.00 | 0.22 | 0.96 | 0.41 | 0.38 | 1.43 | 0.00 | 0.04 | 0.00 | 0.15 | 0.18 |
| DG | 0.00 | 0.00 | 0.00 | 0.93 | 0.00 | 0.00 | 0.31 | 0.77 | 0.31 | 0.00 | 0.00 |
| AMPL | 1.43 | 2.76 | 1.79 | 3.70 | 1.76 | 0.79 | 4.61 | 2.76 | 3.59 | 1.42 | 1.80 |
| PL | 4.91 | 3.13 | 2.75 | 2.93 | 3.59 | 3.31 | 2.08 | 1.72 | 2.70 | 4.10 | 1.89 |
| WE (µg) | 280.0 | 452.4 | 479.7 | 504.2 | 534.7 | 578.4 | 377.6 | 420.8 | 302.8 | 535.3 | 607.3 |
| TL (µg) | 317.4 | 543.8 | 617.7 | 618.6 | 645.9 | 694.8 | 463.5 | 505.2 | 361.9 | 707.2 | 702.2 |
| DM (mg) | -- | -- | -- | -- | 1.19 | 1.38 | 1.22 | 1.22 | 1.22 | 1.33 | 1.39 |

Appendix 4. Continued.

| Lipid Class (%) | 54a | 54b | 58 | 66 | 76 |
|---|---|---|---|---|---|
| HC | 1.15 | 1.56 | 1.43 | 1.46 | 0.94 |
| WE | 87.90 | 88.74 | 90.01 | 80.08 | 91.65 |
| ME | 0.73 | 0.21 | 0.24 | 1.17 | 0.40 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 5.17 | 7.54 | 5.63 | 7.38 | 2.34 |
| FFA | 0.00 | 0.00 | 0.00 | 0.51 | 0.00 |
| ALC | 0.00 | 0.00 | 0.00 | 0.00 | 0.68 |
| ST | 0.00 | 0.00 | 0.00 | 2.43 | 0.00 |
| DG | 0.87 | 0.00 | 0.00 | 0.00 | 0.00 |
| AMPL | 2.13 | 0.81 | 0.79 | 2.53 | 1.65 |
| PL | 2.06 | 1.14 | 1.90 | 4.44 | 2.35 |
| WE (µg) | 561.5 | 626.5 | 493.5 | 588.9 | 598.3 |
| TL (µg) | 638.7 | 565.9 | 548.3 | 735.3 | 652.8 |
| DM (mg) | 1.14 | 1.24 | 1.04 | 1.20 | 1.15 |

Appendix 5. Complete lipid class composition of surface-layer *Metridia longa* CVI-F (NOW; fall 1999).

| Lipid Class (%) | 14 | 45 | 66-A | 66-B | 66-C | 76 |
|---|---|---|---|---|---|---|
| HC | 1.2 | 0.38 | 0.64 | 0.72 | 1.09 | 0.54 |
| WE | 65.62 | 74.72 | 79.69 | 80.32 | 78.04 | 62.28 |
| ME | 0.28 | 0.00 | 0.009 | 0.002 | 0.00 | 4.60 |
| KET | 0.00 | 0.00 | 0.009 | 0.002 | 0.00 | 0.00 |
| TG | 8.88 | 13.59 | 4.24 | 4.66 | 4.00 | 3.58 |
| FFA | 0.00 | 0.13 | 1.11 | 1.37 | 1.49 | 0.66 |
| ALC | 0.00 | 0.00 | 0.00 | 0.007 | 0.71 | 1.27 |
| ST | 1.22 | 0.80 | 0.35 | 0.31 | 0.94 | 3.30 |
| DG | 0.64 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08 |
| AMPL | 6.66 | 3.35 | 1.54 | 1.58 | 1.21 | 4.80 |
| PL | 15.47 | 7.03 | 12.44 | 11.05 | 12.54 | 18.87 |
| | | | | | | |
| WE (μg) | 196.1 | 204.6 | 120.6 | 120.1 | 117.4 | 127.4 |
| TL (μg) | 196.1 | 204.6 | 151.3 | 149.5 | 150.4 | 127.4 |
| DM (mg) | 0.46 | 0.53 | 0.37 | 0.37 | 0.37 | 0.36 |

Appendix 6. Complete lipid class composition of deep-layer *Metridia longa* CVI-F (NOW; fall 1999).

| Lipid Class (%) | 01 | 02 | 03-A | 03-B | 03-C | 06 | 14 | 32 | 35 | 40 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HC | 0.77 | 0.80 | 0.97 | 0.20 | 0.27 | 0.72 | 0.61 | 0.38 | 0.91 | 0.46 | 0.00 |
| WE | 68.45 | 67.01 | 73.49 | 75.47 | 73.89 | 72.61 | 69.23 | 71.37 | 77.42 | 79.28 | 77.37 |
| ME | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.28 | 0.00 | 0.00 | 0.00 | 0.00 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 14.63 | 15.86 | 11.58 | 10.48 | 13.32 | 10.34 | 11.34 | 10.53 | 12.58 | 13.61 | 15.08 |
| FFA | 0.60 | 0.82 | 1.33 | 2.18 | 1.56 | 0.00 | 0.00 | 0.00 | 0.30 | 0.00 | 0.25 |
| ALC | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.00 | 0.05 | 0.00 | 0.00 | 0.00 |
| ST | 0.73 | 1.02 | 0.81 | 0.62 | 0.57 | 0.84 | 1.10 | 1.62 | 0.45 | 0.42 | 0.39 |
| DG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.49 | 0.38 | 0.00 | 0.00 | 0.00 |
| AMPL | 2.91 | 2.60 | 2.22 | 2.10 | 1.48 | 6.26 | 5.63 | 4.41 | 3.60 | 2.33 | 3.74 |
| PL | 11.90 | 11.89 | 9.61 | 8.95 | 8.91 | 9.10 | 11.32 | 11.27 | 4.73 | 3.90 | 3.17 |
| WE (µg) | 104.5 | 98.6 | 101.6 | 111.5 | 109.2 | 135.5 | 138.4 | 152.0 | 104.1 | 113.7 | 126.8 |
| TL (µg) | 152.7 | 147.1 | 138.2 | 147.7 | 147.8 | 186.6 | 199.8 | 213.0 | 134.4 | 143.5 | 163.9 |
| DM (mg) | 0.46 | 0.50 | 0.41 | 0.41 | 0.41 | 0.51 | 0.46 | 0.53 | 0.47 | 0.41 | 0.53 |

Appendix 6. Continued.

| Lipid Class (%) | 54a-A | 54a-B | 54a-C | 54b | 58 | 66 | 76 |
|---|---|---|---|---|---|---|---|
| HC | 0.54 | 0.52 | 0.55 | 0.57 | 0.24 | 0.25 | 0.69 |
| WE | 79.62 | 78.29 | 69.69 | 69.86 | 49.32 | 75.37 | 71.48 |
| ME | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 |
| KET | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| TG | 4.81 | 5.53 | 7.23 | 14.81 | 4.59 | 3.65 | 6.21 |
| FFA | 0.00 | 0.00 | 0.00 | 0.25 | 0.71 | 0.47 | 0.00 |
| ALC | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.71 | 1.17 |
| ST | 0.35 | 0.23 | 0.64 | 0.44 | 2.44 | 1.24 | 0.00 |
| DG | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.24 | 0.17 |
| AMPL | 3.30 | 4.02 | 3.06 | 2.05 | 4.97 | 2.21 | 3.86 |
| PL | 11.37 | 11.40 | 18.84 | 12.00 | 37.73 | 15.86 | 16.22 |
| WE (µg) | -- | 54.6 | 65.8 | 130.2 | 19.9 | 117.7 | 84.6 |
| TL (µg) | -- | 69.7 | 94.5 | 186.4 | 40.3 | 156.2 | 118.3 |
| DM (mg) | 0.40 | 0.40 | 0.40 | 0.49 | 0.37 | 0.46 | 0.45 |

Appendix 7. Complete fatty acid composition of surface-layer *Calanus hyperboreus* CV (NOW; fall 1999); column headings refer to stations; TFA=total fatty acids (μg copepod$^{-1}$).

| Fatty acid (%) | 03 | 06 | 01 | 14-A | 14-B | 14-C | 32 | 35 | 40 | 44 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 2.91 | 2.74 | 2.88 | 2.40 | 2.72 | 2.65 | 2.75 | 3.02 | 2.61 | 2.67 | 2.63 |
| 14:1 | 0.34 | 0.70 | 0.16 | 0.28 | 0.25 | 0.27 | 0.37 | 0.20 | 0.18 | 0.25 | 0.18 |
| i-15:0 | 0.09 | 0.68 | 0.06 | 0.11 | 0.13 | 0.09 | 0.12 | 0.13 | 0.14 | 0.20 | 0.14 |
| ai-15:0 | 0.00 | 0.00 | 0.06 | 0.05 | 0.00 | 0.07 | 0.00 | 0.07 | 0.00 | 0.07 | 0.22 |
| 15:0 | 0.04 | 0.00 | 0.08 | 0.06 | 0.05 | 0.09 | 0.05 | 0.16 | 0.05 | 0.05 | 0.13 |
| 15:1 | 0.05 | 0.00 | 0.09 | 0.08 | 0.07 | 0.12 | 0.09 | 0.30 | 0.06 | 0.07 | 0.12 |
| i-16:0 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 |
| ai-16:0 | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 |
| 16:0 | 2.59 | 2.34 | 3.25 | 2.33 | 2.55 | 2.43 | 2.55 | 3.02 | 2.27 | 2.42 | 2.64 |
| 16:1(n-9) | 0.20 | 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 |
| 16:1(n-7) | 19.65 | 11.75 | 23.56 | 16.81 | 15.02 | 23.08 | 18.37 | 20.49 | 12.99 | 19.02 | 19.98 |
| 16:1(n-5) | 0.19 | 0.13 | 0.25 | 0.19 | 0.20 | 0.23 | 0.17 | 0.53 | 0.17 | 0.18 | 0.23 |
| i-17:0 | 0.00 | 0.00 | 0.03 | 0.05 | 0.00 | 0.03 | 0.00 | 0.14 | 0.05 | 0.04 | 0.00 |
| ai-17:0 | 0.30 | 0.31 | 0.38 | 0.33 | 0.30 | 0.30 | 0.30 | 0.60 | 0.33 | 0.34 | 0.51 |
| 16:2(n-4) | 1.51 | 1.59 | 1.62 | 1.76 | 1.47 | 1.53 | 1.54 | 1.79 | 1.56 | 1.48 | 1.69 |
| 16:2? | 0.06 | 0.00 | 0.10 | 0.00 | 0.04 | 0.05 | 0.03 | 0.24 | 0.00 | 0.00 | 0.08 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 1.70 | 1.70 | 1.68 | 1.91 | 1.38 | 1.79 | 1.29 | 1.81 | 1.97 | 2.69 | 1.54 |
| 17:1 | 0.05 | 0.08 | 0.10 | 0.05 | 0.07 | 0.04 | 0.07 | 0.13 | 0.10 | 0.09 | 0.00 |
| 16:3? | 0.79 | 1.36 | 1.12 | 1.18 | 1.14 | 0.98 | 1.03 | 1.17 | 1.00 | 1.01 | 1.10 |
| 16:4(n-3) | 0.45 | 0.27 | 0.46 | 0.35 | 0.33 | 0.45 | 0.36 | 0.43 | 0.06 | 0.27 | 0.43 |
| 16:4(n-1) | 4.68 | 7.19 | 5.05 | 6.84 | 6.61 | 5.07 | 5.55 | 5.68 | 5.97 | 4.95 | 6.14 |
| 18:0 | 0.16 | 0.13 | 0.19 | 0.14 | 0.12 | 0.17 | 0.18 | 0.14 | 0.14 | 0.15 | 0.10 |
| 18:1(n-9) | 1.41 | 1.02 | 2.04 | 1.60 | 1.32 | 2.22 | 1.49 | 1.69 | 1.29 | 1.60 | 1.57 |
| 18:1(n-7) | 1.18 | 0.88 | 1.25 | 1.19 | 1.06 | 1.12 | 1.15 | 1.14 | 0.97 | 1.50 | 1.13 |
| 18:1(n-5) | 0.35 | 0.39 | 0.47 | 0.00 | 0.46 | 0.41 | 0.40 | 0.41 | 0.37 | 0.46 | 0.38 |
| 18:2(n-6) | 0.57 | 0.39 | 0.64 | 0.51 | 0.44 | 0.65 | 0.52 | 0.59 | 0.54 | 0.68 | 0.47 |
| 18:2(n-4) | 0.06 | 0.12 | 0.13 | 0.18 | 0.19 | 0.16 | 0.12 | 0.17 | 0.13 | 0.23 | 0.17 |
| 18:3(n-6) | 0.37 | 0.16 | 0.51 | 0.29 | 0.23 | 0.41 | 0.23 | 0.34 | 0.20 | 0.34 | 0.31 |
| 18:3(n-4) | 0.08 | 0.03 | 0.21 | 0.05 | 0.06 | 0.04 | 0.03 | 0.07 | 0.06 | 0.07 | 0.11 |
| 18:3(n-3) | 0.21 | 0.22 | 0.20 | 0.21 | 0.14 | 0.19 | 0.19 | 0.20 | 0.23 | 0.21 | 0.23 |
| 18:4(n-3) | 1.29 | 1.14 | 1.88 | 1.74 | 1.18 | 1.60 | 1.40 | 1.61 | 1.97 | 0.07 | 1.67 |
| 18:4(n-1) | 0.57 | 0.58 | 0.71 | 0.88 | 0.65 | 0.62 | 0.58 | 0.78 | 0.64 | 0.56 | 0.78 |
| 20:0 | 0.04 | 0.04 | 0.14 | 0.05 | 0.10 | 0.05 | 0.07 | 0.04 | 0.26 | 0.03 | 0.00 |
| 20:1(n-11) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:1(n-9) | 6.16 | 4.67 | 8.12 | 7.27 | 7.32 | 8.32 | 5.47 | 7.04 | 5.98 | 4.73 | 7.47 |
| 20:1(n-7) | 0.65 | 0.40 | 1.46 | 0.67 | 0.87 | 1.02 | 1.05 | 1.06 | 0.71 | 0.65 | 1.39 |
| 20:2(n-6) | 0.04 | 0.00 | 0.23 | 0.00 | 0.05 | 0.06 | 0.04 | 0.08 | 0.07 | 0.08 | 0.09 |
| 20:3(n-6) | 0.08 | 0.03 | 0.24 | 0.07 | 0.06 | 0.11 | 0.07 | 0.11 | 0.13 | 0.11 | 0.06 |
| 20:4(n-6) | 0.53 | 0.41 | 0.61 | 0.40 | 0.43 | 0.58 | 0.47 | 0.57 | 0.58 | 0.81 | 0.50 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.13 |
| 20:4(n-3) | 0.44 | 0.33 | 0.48 | 0.42 | 0.44 | 0.48 | 0.40 | 0.48 | 0.43 | 0.51 | 0.43 |
| 20:5(n-3) | 33.73 | 42.71 | 26.12 | 35.21 | 33.82 | 27.00 | 34.83 | 30.86 | 42.13 | 39.57 | 32.31 |
| 22:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 22:1(n-11) | 6.56 | 4.07 | 6.60 | 7.09 | 7.19 | 8.13 | 5.77 | 5.94 | 5.41 | 4.45 | 5.62 |
| 22:1(n-9) | 0.90 | 0.67 | 1.27 | 1.23 | 1.34 | 1.49 | 0.83 | 1.11 | 1.43 | 1.08 | 1.45 |
| 22:1(n-7) | 0.17 | 0.07 | 0.23 | 0.17 | 0.19 | 0.27 | 0.14 | 0.22 | 0.16 | 0.20 | 0.23 |
| 21:5(n-3) | 0.05 | 0.07 | 0.09 | 0.15 | 0.10 | 0.06 | 0.10 | 0.11 | 0.08 | 0.07 | 0.09 |
| 22:5(n-3) | 2.21 | 4.75 | 1.77 | 2.06 | 2.85 | 2.15 | 1.69 | 1.94 | 2.07 | 2.19 | 2.16 |
| 22:6(n-3) | 6.08 | 5.60 | 3.14 | 3.35 | 5.71 | 3.08 | 7.20 | 2.95 | 4.21 | 3.60 | 2.99 |
| 24:1 | 0.50 | 0.12 | 0.21 | 0.27 | 1.33 | 0.32 | 0.95 | 0.31 | 0.29 | 0.20 | 0.29 |
| TFA (μg) | 569.5 | 513.4 | 786.0 | 381.6 | 639.3 | 414.3 | 517.3 | 450.7 | 690.9 | 386.1 | 733.5 |

Appendix 7. Continued.

| Fatty acid (%) | 50 | 54a | 54b | 58 | 66 | 68 |
|---|---|---|---|---|---|---|
| 14:0 | 4.17 | 4.31 | 2.71 | 3.45 | 2.63 | 3.33 |
| 14:1 | 0.29 | 0.31 | 0.20 | 0.19 | 0.30 | 0.25 |
| i-15:0 | 0.12 | 0.19 | 0.13 | 0.14 | 0.11 | 0.16 |
| ai-15:0 | 0.00 | 0.13 | 0.10 | 0.13 | 0.04 | 0.04 |
| 15:0 | 0.16 | 0.32 | 0.00 | 0.19 | 0.07 | 0.09 |
| 15:1 | 0.16 | 0.00 | 0.08 | 0.00 | 0.09 | 0.12 |
| i-16:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 |
| ai-16:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0 | 3.89 | 5.04 | 2.49 | 4.26 | 2.82 | 3.19 |
| 16:1(n-9) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 |
| 16:1(n-7) | 29.66 | 13.83 | 16.76 | 11.09 | 13.81 | 19.88 |
| 16:1(n-5) | 0.23 | 0.51 | 0.23 | 0.48 | 0.16 | 0.30 |
| i-17:0 | 0.00 | 0.18 | 0.00 | 0.13 | 0.00 | 0.10 |
| ai-17:0 | 0.32 | 0.29 | 0.36 | 0.27 | 0.29 | 0.27 |
| 16:2(n-4) | 1.55 | 1.19 | 1.80 | 1.26 | 1.60 | 1.44 |
| 16:2? | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 1.14 | 1.37 | 2.03 | 1.20 | 2.17 | 1.42 |
| 17:1 | 0.00 | 0.00 | 0.10 | 0.14 | 0.05 | 0.06 |
| 16:3? | 0.53 | 0.70 | 0.99 | 0.72 | 1.37 | 0.90 |
| 16:4(n-3) | 0.00 | 0.35 | 0.24 | 0.00 | 0.17 | 0.23 |
| 16:4(n-1) | 2.68 | 2.28 | 5.14 | 1.60 | 5.15 | 4.03 |
| 18:0 | 0.64 | 0.71 | 0.14 | 0.39 | 0.50 | 0.21 |
| 18:1(n-9) | 1.95 | 3.03 | 1.61 | 3.91 | 1.47 | 2.27 |
| 18:1(n-7) | 1.57 | 1.33 | 1.17 | 1.35 | 1.09 | 1.29 |
| 18:1(n-5) | 0.45 | 0.70 | 0.36 | 0.74 | 0.45 | 0.47 |
| 18:2(n-6) | 0.72 | 1.99 | 0.63 | 3.09 | 0.55 | 1.33 |
| 18:2(n-4) | 0.14 | 0.22 | 0.18 | 0.41 | 0.33 | 0.16 |
| 18:3(n-6) | 0.68 | 0.21 | 0.26 | 0.32 | 0.35 | 0.27 |
| 18:3(n-4) | 0.00 | 0.09 | 0.00 | 0.00 | 0.19 | 0.06 |
| 18:3(n-3) | 0.25 | 1.07 | 0.24 | 1.85 | 0.58 | 0.62 |
| 18:4(n-3) | 1.30 | 7.49 | 2.21 | 9.73 | 1.72 | 4.57 |
| 18:4(n-1) | 0.52 | 0.40 | 0.70 | 0.53 | 0.68 | 0.56 |
| 20:0 | 1.71 | 1.69 | 0.00 | 0.00 | 0.33 | 0.15 |
| 20:1(n-11) | 0.88 | 0.27 | 0.00 | 0.00 | 0.09 | 0.00 |
| 20:1(n-9) | 6.92 | 10.04 | 7.19 | 10.18 | 5.60 | 8.96 |
| 20:1(n-7) | 1.50 | 0.78 | 0.94 | 1.41 | 0.84 | 1.21 |
| 20:2(n-6) | 0.14 | 0.18 | 0.09 | 0.34 | 0.00 | 0.16 |
| 20:3(n-6) | 0.58 | 0.00 | 0.16 | 0.00 | 0.00 | 0.00 |
| 20:4(n-6) | 0.00 | 0.39 | 0.62 | 0.87 | 0.00 | 2.18 |
| 20:3 (n-3) | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-3) | 0.00 | 0.84 | 0.52 | 0.00 | 0.40 | 0.49 |
| 20:5(n-3) | 20.02 | 16.80 | 33.17 | 12.40 | 30.33 | 25.31 |
| 22:0 | 0.00 | 0.92 | 0.00 | 0.14 | 0.02 | 0.01 |
| 22:1(n-11) | 8.51 | 8.38 | 7.85 | 12.98 | 5.22 | 7.19 |
| 22:1(n-9) | 1.21 | 1.79 | 1.95 | 2.39 | 0.79 | 1.48 |
| 22:1(n-7) | 0.48 | 0.26 | 0.24 | 0.36 | 0.14 | 0.20 |
| 21:5(n-3) | 0.46 | 0.24 | 0.10 | 0.21 | 0.00 | 0.11 |
| 22:5(n-3) | 1.20 | 1.10 | 1.73 | 1.06 | 7.57 | 0.00 |
| 22:6(n-3) | 2.57 | 7.37 | 4.18 | 9.68 | 9.90 | 4.59 |
| 24:1 | 0.69 | 0.62 | 0.38 | 0.37 | 0.00 | 0.26 |
| TFA (µg) | 693.2 | 427.8 | -- | 434.4 | 486.9 | 447.8 |

Appendix 8. Complete fatty acid composition of deep-layer *Calanus hyperboreus* CV (NOW: fall 1999).

| Fatty acid (%) | 02-A | 02-B | 02-C | 03 | 06 | 14 | 32 | 35 | 45 | 54a | 54b |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 2.92 | 3.10 | 2.73 | 2.76 | 3.02 | 2.50 | 2.80 | 3.77 | 3.16 | 3.00 | 3.21 |
| 14:1 | 0.19 | 0.19 | 0.17 | 0.27 | 0.25 | 0.46 | 0.33 | 0.26 | 0.30 | 0.20 | 0.37 |
| i-15:0 | 0.08 | 0.13 | 0.14 | 0.08 | 0.12 | 0.18 | 0.19 | 0.09 | 0.10 | 0.09 | 0.12 |
| ai-15:0 | 0.00 | 0.09 | 0.00 | 0.00 | 0.03 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.16 |
| 15:0 | 0.06 | 0.09 | 0.10 | 0.06 | 0.06 | 0.05 | 0.06 | 0.09 | 0.07 | 0.07 | 0.08 |
| 15:1 | 0.10 | 0.11 | 0.14 | 0.10 | 0.08 | 0.05 | 0.10 | 0.00 | 0.11 | 0.07 | 0.26 |
| i-16:0 | 0.00 | 0.00 | 0.08 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 |
| ai-16:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0 | 2.84 | 2.76 | 2.57 | 3.25 | 2.95 | 2.51 | 2.54 | 3.31 | 2.93 | 2.56 | 3.57 |
| 16:1(n-9) | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.23 | 0.03 | 0.15 |
| 16:1(n-7) | 21.61 | 18.15 | 17.31 | 23.20 | 22.38 | 20.01 | 20.82 | 28.75 | 26.27 | 19.43 | 24.09 |
| 16:1(n-5) | 0.16 | 0.26 | 0.19 | 0.15 | 0.15 | 0.20 | 0.19 | 0.36 | 0.23 | 0.21 | 0.40 |
| i-17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 | 0.03 |
| ai-17:0 | 0.30 | 0.36 | 0.30 | 0.31 | 0.26 | 0.36 | 0.32 | 0.36 | 0.29 | 0.24 | 0.34 |
| 16:2(n-4) | 1.55 | 1.49 | 1.57 | 1.67 | 1.33 | 1.49 | 1.51 | 1.37 | 1.51 | 1.33 | 1.81 |
| 16:2? | 0.00 | 0.00 | 0.00 | 0.07 | 0.20 | 0.04 | 0.04 | 0.11 | 0.00 | 0.00 | 0.00 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 1.66 | 1.49 | 1.43 | 1.98 | 1.54 | 1.72 | 1.51 | 1.35 | 1.32 | 1.47 | 2.11 |
| 17:1 | 0.25 | 0.09 | 0.10 | 0.06 | 0.07 | 0.08 | 0.07 | 0.00 | 0.13 | 0.06 | 0.08 |
| 16:3? | 0.99 | 1.06 | 1.00 | 1.24 | 0.89 | 0.89 | 1.05 | 0.63 | 0.82 | 0.65 | 1.51 |
| 16:4(n-3) | 0.46 | 0.40 | 0.35 | 0.25 | 0.18 | 0.27 | 0.29 | 0.33 | 0.27 | 0.21 | 0.16 |
| 16:4(n-1) | 5.51 | 6.28 | 5.71 | 5.27 | 4.75 | 4.54 | 5.11 | 2.54 | 4.53 | 3.45 | 5.68 |
| 18:0 | 0.59 | 0.20 | 0.15 | 0.13 | 0.12 | 0.11 | 0.11 | 0.15 | 0.12 | 0.15 | 0.23 |
| 18:1(n-9) | 2.41 | 1.95 | 1.81 | 1.91 | 1.67 | 1.79 | 1.67 | 1.94 | 2.76 | 1.80 | 1.84 |
| 18:1(n-7) | 1.34 | 1.21 | 1.08 | 1.15 | 1.26 | 1.02 | 1.20 | 1.38 | 1.52 | 1.24 | 1.15 |
| 18:1(n-5) | 0.55 | 0.43 | 0.38 | 0.55 | 0.49 | 0.54 | 0.47 | 0.49 | 0.49 | 0.41 | 0.63 |
| 18:2(n-6) | 0.62 | 0.62 | 0.52 | 0.56 | 0.58 | 0.67 | 0.65 | 1.23 | 0.73 | 0.90 | 1.03 |
| 18:2(n-4) | 0.13 | 0.16 | 0.12 | 0.00 | 0.16 | 0.16 | 0.15 | 0.19 | 0.19 | 0.19 | 0.39 |
| 18:3(n-6) | 0.50 | 0.40 | 0.37 | 0.62 | 0.27 | 0.45 | 0.23 | 0.53 | 0.30 | 0.29 | 0.28 |
| 18:3(n-4) | 0.00 | 0.00 | 0.00 | 0.05 | 0.06 | 0.04 | 0.06 | 0.00 | 0.05 | 0.06 | 0.06 |
| 18:3(n-3) | 0.18 | 0.09 | 0.21 | 0.21 | 0.16 | 0.16 | 0.15 | 0.41 | 0.23 | 0.32 | 0.30 |
| 18:4(n-3) | 1.88 | 2.11 | 1.53 | 1.90 | 1.74 | 1.65 | 1.30 | 4.76 | 2.00 | 3.78 | 2.36 |
| 18:4(n-1) | 0.61 | 0.85 | 0.67 | 0.71 | 0.62 | 0.54 | 0.62 | 0.55 | 0.62 | 0.48 | 0.87 |
| 20:0 | 0.31 | 0.00 | 0.16 | 0.07 | 0.07 | 0.50 | 0.06 | 0.50 | 0.09 | 0.77 | 0.44 |
| 20:1(n-11) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.00 |
| 20:1(n-9) | 5.74 | 8.34 | 5.66 | 7.24 | 7.43 | 7.60 | 5.91 | 4.94 | 6.61 | 7.59 | 4.19 |
| 20:1(n-7) | 1.16 | 1.01 | 0.67 | 0.94 | 1.53 | 1.23 | 0.97 | 1.75 | 1.49 | 1.15 | 0.86 |
| 20:2(n-6) | 0.15 | 0.00 | 0.00 | 0.04 | 0.08 | 0.05 | 0.12 | 0.16 | 0.10 | 0.11 | 0.08 |
| 20:3(n-6) | 0.10 | 0.11 | 0.09 | 0.14 | 0.09 | 0.10 | 0.08 | 0.16 | 0.09 | 0.08 | 0.08 |
| 20:4(n-6) | 0.74 | 0.52 | 0.50 | 0.82 | 0.47 | 0.54 | 0.53 | 0.72 | 0.48 | 0.49 | 0.43 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.03 | 0.01 | 0.11 | 0.00 | 0.08 | 0.04 |
| 20:4(n-3) | 0.43 | 0.46 | 3.17 | 0.51 | 0.40 | 0.33 | 0.39 | 0.59 | 0.63 | 2.13 | 0.33 |
| 20:5(n-3) | 27.54 | 30.45 | 35.81 | 24.39 | 27.56 | 26.09 | 31.11 | 20.22 | 25.12 | 26.17 | 29.99 |
| 22:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 22:1(n-11) | 6.96 | 6.27 | 5.12 | 5.88 | 6.67 | 8.07 | 5.05 | 6.48 | 7.34 | 6.26 | 3.70 |
| 22:1(n-9) | 2.24 | 1.98 | 1.61 | 1.18 | 1.93 | 1.59 | 1.07 | 1.79 | 1.45 | 1.53 | 0.47 |
| 22:1(n-7) | 0.29 | 0.23 | 0.23 | 0.18 | 0.32 | 0.32 | 0.18 | 0.36 | 0.28 | 0.25 | 0.00 |
| 21:5(n-3) | 0.00 | 0.12 | 0.00 | 0.10 | 0.11 | 0.50 | 0.31 | 0.00 | 0.00 | 1.84 | 0.17 |
| 22:5(n-3) | 1.92 | 1.72 | 1.74 | 1.92 | 1.94 | 1.66 | 2.55 | 1.50 | 1.64 | 2.07 | 1.83 |
| 22:6(n-3) | 4.47 | 4.36 | 4.29 | 7.42 | 5.58 | 7.92 | 7.48 | 5.42 | 3.11 | 5.81 | 3.76 |
| 24:1 | 0.31 | 0.34 | 0.24 | 0.67 | 0.34 | 0.95 | 0.61 | 0.38 | 0.32 | 0.93 | 0.34 |
| TFA (µg) | 393.9 | 707.8 | 653.7 | 983.2 | 989.7 | 744.8 | 860.1 | 1118.6 | 794.9 | 485.4 | 438.3 |

Appendix 8. Continued.

| Fatty acid (%) | 58 | 66 | 76-A | 76-B | 76-C |
|---|---|---|---|---|---|
| 14:0 | 4.64 | 3.31 | 3.48 | 3.52 | 4.05 |
| 14:1 | 0.34 | 0.26 | 0.30 | 0.22 | 0.31 |
| i-15:0 | 0.23 | 0.07 | 0.13 | 0.10 | 0.14 |
| ai-15:0 | 0.31 | 0.00 | 0.10 | 0.09 | 0.16 |
| 15:0 | 0.40 | 0.09 | 0.19 | 0.14 | 0.34 |
| 15:1 | 0.14 | 0.09 | 0.11 | 0.10 | 0.23 |
| i-16:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 |
| ai-16:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0 | 5.27 | 3.50 | 3.69 | 3.53 | 4.46 |
| 16:1(n-9) | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:1(n-7) | 22.63 | 28.51 | 16.99 | 16.60 | 15.25 |
| 16:1(n-5) | 0.57 | 0.18 | 0.55 | 0.47 | 0.57 |
| i-17:0 | 0.14 | 0.01 | 0.19 | 0.16 | 0.20 |
| ai-17:0 | 0.23 | 0.25 | 0.19 | 0.23 | 0.18 |
| 16:2(n-4) | 1.27 | 1.34 | 0.87 | 1.02 | 0.76 |
| 16:2? | 0.00 | 0.10 | 0.00 | 0.05 | 0.00 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 0.73 | 1.44 | 0.74 | 0.75 | 0.72 |
| 17:1 | 0.11 | 0.00 | 0.00 | 0.08 | 0.00 |
| 16:3? | 0.88 | 0.72 | 0.57 | 0.73 | 0.54 |
| 16:4(n-3) | 0.55 | 0.26 | 0.36 | 0.35 | 0.42 |
| 16:4(n-1) | 1.62 | 2.76 | 0.73 | 1.54 | 0.83 |
| 18:0 | 0.38 | 0.43 | 0.29 | 0.23 | 0.30 |
| 18:1(n-9) | 3.89 | 1.96 | 3.37 | 3.00 | 3.37 |
| 18:1(n-7) | 1.79 | 1.38 | 1.34 | 1.38 | 1.40 |
| 18:1(n-5) | 1.00 | 0.47 | 0.72 | 0.67 | 0.73 |
| 18:2(n-6) | 3.53 | 0.92 | 2.31 | 2.13 | 2.80 |
| 18:2(n-4) | 0.34 | 0.14 | 0.36 | 0.34 | 0.35 |
| 18:3(n-6) | 0.45 | 0.52 | 0.32 | 0.47 | 0.36 |
| 18:3(n-4) | 0.00 | 0.06 | 0.00 | 0.04 | 0.11 |
| 18:3(n-3) | 1.58 | 0.29 | 1.70 | 1.35 | 1.77 |
| 18:4(n-3) | 10.09 | 2.60 | 11.45 | 9.57 | 11.08 |
| 18:4(n-1) | 0.39 | 0.41 | 0.28 | 0.47 | 0.39 |
| 20:0 | 0.60 | 0.25 | 0.00 | 0.04 | 0.00 |
| 20:1(n-11) | 0.00 | 0.06 | 0.38 | 0.33 | 0.32 |
| 20:1(n-9) | 3.14 | 6.80 | 11.60 | 11.93 | 11.84 |
| 20:1(n-7) | 0.16 | 1.77 | 1.09 | 1.30 | 0.99 |
| 20:2(n-6) | 0.36 | 0.11 | 0.27 | 0.27 | 0.35 |
| 20:3(n-6) | 0.00 | 0.13 | 0.12 | 0.15 | 0.14 |
| 20:4(n-6) | 0.27 | 0.46 | 0.44 | 0.38 | 0.39 |
| 20:3 (n-3) | 0.14 | 0.03 | 0.14 | 0.13 | 0.14 |
| 20:4(n-3) | 1.06 | 0.49 | 1.32 | 1.11 | 0.00 |
| 20:5(n-3) | 10.91 | 17.51 | 10.60 | 12.04 | 10.69 |
| 22:0 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| 22:1(n-11) | 7.97 | 5.35 | 7.87 | 8.05 | 7.87 |
| 22:1(n-9) | 1.90 | 1.75 | 1.98 | 3.06 | 2.68 |
| 22:1(n-7) | 0.24 | 0.30 | 0.21 | 0.30 | 0.21 |
| 21:5(n-3) | 0.22 | 0.47 | 0.34 | 0.21 | 0.27 |
| 22:5(n-3) | 1.06 | 2.79 | 1.02 | 1.30 | 1.13 |
| 22:6(n-3) | 7.90 | 8.21 | 10.83 | 9.42 | 10.61 |
| 24:1 | 0.44 | 1.43 | 0.49 | 0.65 | 0.48 |
| TFA (µg) | 453.8 | 1132.3 | 577.9 | 898.4 | 642.1 |

Appendix 9. Complete fatty acid composition of surface-layer *Calanus glacialis* CV (NOW: fall 1999).

| Fatty acid (%) | 03 | 06 | 01 | 14 | 32 | 35 | 40 | 44 | 45 | 54a |
|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 7.86 | 7.81 | 8.24 | 7.02 | 8.12 | 8.93 | 7.64 | 9.11 | 8.15 | 10.45 |
| 14:1 | 0.32 | 0.26 | 0.11 | 0.21 | 0.14 | 0.15 | 0.14 | 0.19 | 0.12 | 0.38 |
| i-15:0 | 0.13 | 0.18 | 0.15 | 0.16 | 0.19 | 0.26 | 0.22 | 0.25 | 0.20 | 0.37 |
| ai-15:0 | 0.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.08 | 0.06 | 0.04 | 0.08 | 0.31 |
| 15:0 | 0.15 | 0.16 | 0.16 | 0.15 | 0.18 | 0.15 | 0.19 | 0.22 | 0.21 | 0.79 |
| 15:1 | 0.12 | 0.18 | 0.15 | 0.16 | 0.28 | 0.13 | 0.32 | 0.28 | 0.25 | 0.78 |
| i-16:0 | 0.00 | 0.00 | 0.02 | 0.00 | 0.08 | 0.05 | 0.15 | 0.11 | 0.08 | 0.45 |
| ai-16:0 | 0.00 | 0.00 | 0.02 | 0.03 | 0.00 | 0.00 | 0.10 | 0.07 | 0.06 | 0.32 |
| 16:0 | 5.32 | 5.48 | 5.84 | 5.61 | 5.92 | 5.73 | 4.93 | 6.23 | 5.53 | 8.50 |
| 16:1(n-9) | 0.25 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:1(n-7) | 18.44 | 13.18 | 22.57 | 15.44 | 19.13 | 14.72 | 12.87 | 19.81 | 20.25 | 20.53 |
| 16:1(n-5) | 0.33 | 0.31 | 0.43 | 0.32 | 0.42 | 0.45 | 0.43 | 0.50 | 0.54 | 0.85 |
| i-17:0 | 0.00 | 0.07 | 0.07 | 0.05 | 0.01 | 0.07 | 0.09 | 0.18 | 0.09 | 0.32 |
| ai-17:0 | 0.30 | 0.36 | 0.36 | 0.30 | 0.35 | 0.45 | 0.55 | 0.70 | 0.46 | 0.45 |
| 16:2(n-4) | 0.83 | 0.94 | 1.05 | 0.73 | 0.83 | 0.75 | 0.87 | 1.16 | 0.96 | 1.11 |
| 16:2? | 0.00 | 0.00 | 0.10 | 0.03 | 0.03 | 0.10 | 0.19 | 0.14 | 0.20 | 0.00 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.17 | 0.00 | 0.10 |
| 16:3(n-4) | 1.69 | 1.67 | 1.49 | 1.16 | 1.10 | 1.75 | 1.93 | 1.65 | 1.25 | 1.32 |
| 17:1 | 0.04 | 0.06 | 0.08 | 0.08 | 0.06 | 0.11 | 0.16 | 0.21 | 0.16 | 0.00 |
| 16:3? | 0.23 | 0.55 | 0.32 | 0.39 | 0.20 | 0.22 | 0.31 | 0.30 | 0.29 | 0.25 |
| 16:4(n-3) | 0.53 | 0.42 | 0.36 | 0.44 | 0.34 | 0.34 | 0.44 | 0.50 | 0.52 | 0.38 |
| 16:4(n-1) | 5.84 | 6.98 | 5.18 | 5.61 | 4.91 | 5.44 | 5.18 | 4.99 | 4.72 | 2.09 |
| 18:0 | 0.73 | 0.24 | 0.24 | 0.29 | 0.16 | 0.26 | 0.22 | 0.29 | 0.24 | 0.37 |
| 18:1(n-9) | 1.58 | 1.63 | 2.08 | 1.57 | 1.43 | 1.69 | 1.75 | 1.86 | 1.61 | 4.58 |
| 18:1(n-7) | 0.49 | 0.62 | 0.90 | 0.62 | 0.67 | 0.77 | 0.63 | 0.84 | 0.65 | 1.03 |
| 18:1(n-5) | 0.26 | 0.37 | 0.30 | 0.32 | 0.28 | 0.30 | 0.29 | 0.31 | 0.26 | 0.56 |
| 18:2(n-6) | 0.40 | 0.34 | 0.39 | 0.31 | 0.35 | 0.34 | 0.36 | 0.39 | 0.36 | 1.31 |
| 18:2(n-4) | 0.00 | 0.06 | 0.10 | 0.07 | 0.06 | 0.09 | 0.10 | 0.06 | 0.09 | 0.42 |
| 18:3(n-6) | 0.38 | 0.23 | 0.37 | 0.27 | 0.28 | 0.23 | 0.23 | 0.30 | 0.29 | 0.29 |
| 18:3(n-4) | 0.00 | 0.00 | 0.03 | 0.05 | 0.03 | 0.00 | 0.04 | 0.00 | 0.02 | 0.00 |
| 18:3(n-3) | 0.14 | 0.17 | 0.13 | 0.16 | 0.14 | 0.14 | 0.15 | 0.13 | 0.11 | 0.74 |
| 18:4(n-3) | 1.97 | 2.27 | 1.75 | 1.54 | 1.74 | 1.84 | 2.06 | 1.73 | 2.11 | 7.63 |
| 18:4(n-1) | 0.29 | 0.39 | 0.34 | 0.25 | 0.23 | 0.28 | 0.33 | 0.27 | 0.26 | 0.24 |
| 20:0 | 0.03 | 0.14 | 0.06 | 0.28 | 0.07 | 0.10 | 0.06 | 0.06 | 0.05 | 0.00 |
| 20:1(n-11) | 0.00 | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.35 |
| 20:1(n-9) | 8.61 | 6.65 | 8.65 | 4.63 | 5.19 | 6.87 | 6.92 | 6.76 | 5.58 | 9.43 |
| 20:1(n-7) | 0.12 | 0.10 | 0.27 | 0.23 | 0.06 | 0.16 | 0.15 | 0.15 | 0.15 | 0.17 |
| 20:2(n-6) | 0.00 | 0.00 | 0.03 | 0.01 | 0.01 | 0.11 | 0.00 | 0.00 | 0.02 | 0.10 |
| 20:3(n-6) | 0.07 | 0.05 | 0.07 | 0.04 | 0.07 | 0.07 | 0.06 | 0.09 | 0.06 | 0.00 |
| 20:4(n-6) | 0.53 | 0.34 | 0.44 | 0.45 | 0.44 | 0.60 | 0.54 | 0.54 | 0.41 | 0.37 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-3) | 0.36 | 0.32 | 0.35 | 0.28 | 0.34 | 0.37 | 0.35 | 0.38 | 0.34 | 0.69 |
| 20:5(n-3) | 29.77 | 32.70 | 26.98 | 29.34 | 33.09 | 35.18 | 36.88 | 30.41 | 33.53 | 10.58 |
| 22:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.06 | 0.00 |
| 22:1(n-11) | 5.08 | 5.01 | 5.51 | 3.12 | 3.66 | 5.81 | 4.82 | 4.42 | 3.91 | 5.86 |
| 22:1(n-9) | 0.53 | 0.44 | 0.45 | 0.40 | 0.47 | 0.42 | 0.95 | 0.36 | 0.94 | 0.45 |
| 22:1(n-7) | 0.09 | 0.06 | 0.10 | 0.05 | 0.06 | 0.08 | 0.09 | 0.05 | 0.11 | 0.10 |
| 21:5(n-3) | 0.43 | 0.34 | 0.37 | 1.45 | 0.86 | 0.34 | 0.31 | 0.31 | 0.31 | 0.57 |
| 22:5(n-3) | 1.22 | 1.52 | 0.95 | 3.71 | 1.66 | 1.09 | 1.08 | 0.94 | 0.93 | 0.53 |
| 22:6(n-3) | 3.93 | 6.59 | 2.09 | 10.99 | 5.60 | 2.68 | 4.59 | 2.32 | 3.15 | 3.55 |
| 24:1 | 0.63 | 0.67 | 0.27 | 1.62 | 0.77 | 0.28 | 0.28 | 0.21 | 0.29 | 0.36 |
| TFA (µg) | 340.5 | 179.5 | 333.9 | 255.2 | 414.5 | 203.9 | 351.5 | 197.3 | 297.7 | 128.5 |

Appendix 9. Continued.

| Fatty acid (%) | 54b-A | 54b-B | 54b-C | 66 | 68 | 76 |
|---|---|---|---|---|---|---|
| 14:0 | 7.74 | 8.25 | 8.90 | 8.42 | 8.30 | 9.46 |
| 14:1 | 0.15 | 0.00 | 0.00 | 0.18 | 0.17 | 0.50 |
| i-15:0 | 0.21 | 0.19 | 0.13 | 0.24 | 0.00 | 0.34 |
| ai-15:0 | 0.08 | 0.05 | 0.05 | 0.07 | 0.58 | 0.12 |
| 15:0 | 0.19 | 0.13 | 0.19 | 0.20 | 0.27 | 0.42 |
| 15:1 | 0.18 | 0.09 | 0.16 | 0.16 | 0.21 | 0.15 |
| i-16:0 | 0.05 | 0.04 | 0.04 | 0.05 | 0.00 | 0.05 |
| ai-16:0 | 0.00 | 0.04 | 0.02 | 0.00 | 0.00 | 0.16 |
| 16:0 | 5.91 | 5.19 | 5.23 | 5.62 | 6.49 | 8.33 |
| 16:1(n-9) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 |
| 16:1(n-7) | 14.47 | 14.23 | 14.47 | 14.43 | 18.11 | 19.64 |
| 16:1(n-5) | 0.38 | 0.00 | 0.00 | 0.40 | 0.39 | 0.66 |
| i-17:0 | 0.03 | 0.00 | 0.00 | 0.06 | 0.00 | 0.10 |
| ai-17:0 | 0.38 | 0.44 | 0.41 | 0.44 | 0.41 | 0.20 |
| 16:2(n-4) | 0.87 | 0.83 | 0.92 | 0.94 | 0.82 | 0.93 |
| 16:2? | 0.08 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 1.89 | 1.81 | 1.91 | 2.20 | 2.10 | 1.10 |
| 17:1 | 0.11 | 0.00 | 0.00 | 0.05 | 0.05 | 0.00 |
| 16:3? | 0.28 | 0.31 | 0.24 | 0.32 | 0.27 | 0.26 |
| 16:4(n-3) | 0.33 | 0.54 | 0.28 | 0.33 | 0.36 | 0.36 |
| 16:4(n-1) | 5.19 | 5.87 | 5.32 | 5.80 | 5.36 | 1.15 |
| 18:0 | 0.26 | 0.30 | 0.22 | 0.29 | 0.24 | 0.63 |
| 18:1(n-9) | 1.92 | 1.91 | 2.12 | 1.71 | 2.42 | 4.46 |
| 18:1(n-7) | 0.78 | 0.63 | 0.62 | 0.65 | 0.69 | 0.91 |
| 18:1(n-5) | 0.33 | 0.34 | 0.30 | 0.31 | 0.59 | 0.47 |
| 18:2(n-6) | 0.45 | 0.41 | 0.48 | 0.42 | 0.57 | 1.44 |
| 18:2(n-4) | 0.12 | 0.17 | 0.13 | 0.08 | 0.05 | 0.60 |
| 18:3(n-6) | 0.31 | 0.36 | 0.31 | 0.26 | 0.28 | 0.46 |
| 18:3(n-4) | 0.06 | 0.12 | 0.05 | 0.05 | 0.04 | 0.31 |
| 18:3(n-3) | 0.19 | 0.22 | 0.21 | 0.17 | 0.20 | 0.99 |
| 18:4(n-3) | 2.68 | 1.97 | 2.92 | 2.26 | 2.31 | 6.35 |
| 18:4(n-1) | 0.32 | 0.46 | 0.34 | 0.33 | 0.48 | 0.43 |
| 20:0 | 0.08 | 0.15 | 0.07 | 0.29 | 0.14 | 0.27 |
| 20:1(n-11) | 0.08 | 0.06 | 0.04 | 0.03 | 0.00 | 0.26 |
| 20:1(n-9) | 7.23 | 7.35 | 8.40 | 6.32 | 6.99 | 12.48 |
| 20:1(n-7) | 0.46 | 0.21 | 0.15 | 0.32 | 0.15 | 0.27 |
| 20:2(n-6) | 0.00 | 0.05 | 0.03 | 0.02 | 0.18 | 0.83 |
| 20:3(n-6) | 0.00 | 0.07 | 0.07 | 0.09 | 0.08 | 0.08 |
| 20:4(n-6) | 0.56 | 0.53 | 0.50 | 0.64 | 0.67 | 0.33 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.03 | 0.00 | 0.00 | 0.05 |
| 20:4(n-3) | 0.41 | 0.35 | 0.40 | 0.36 | 0.41 | 0.69 |
| 20:5(n-3) | 32.21 | 33.86 | 30.84 | 31.34 | 31.61 | 9.92 |
| 22:0 | 0.03 | 0.03 | 0.03 | 0.00 | 0.00 | 0.00 |
| 22:1(n-11) | 5.20 | 5.52 | 6.35 | 4.99 | 3.66 | 4.70 |
| 22:1(n-9) | 0.51 | 0.59 | 0.54 | 0.50 | 0.45 | 0.71 |
| 22:1(n-7) | 0.08 | 0.07 | 0.08 | 0.08 | 0.06 | 0.14 |
| 21:5(n-3) | 0.37 | 0.36 | 0.40 | 0.28 | 0.30 | 0.31 |
| 22:5(n-3) | 1.12 | 0.99 | 1.06 | 0.94 | 0.00 | 0.80 |
| 22:6(n-3) | 5.42 | 4.49 | 4.73 | 5.74 | 3.31 | 6.08 |
| 24:1 | 0.28 | 0.29 | 0.29 | 1.64 | 0.24 | 1.06 |
| TFA (µg) | 244.5 | 307.6 | 316.9 | 243.9 | 154.0 | 279.6 |

M

Appendix 10. Complete fatty acid composition of deep-layer *Calanus glacialis* CV (NOW; fall 1999).

| Fatty acid (%) | 02-A | 02-B | 03 | 06 | 14 | 32-A | 32-B | 32-C | 35 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 9.45 | 9.18 | 8.20 | 7.76 | 8.20 | 8.07 | 8.30 | 8.29 | 8.77 | 8.08 |
| 14:1 | 0.17 | 0.17 | 0.34 | 0.15 | 0.31 | 0.13 | 0.00 | 0.44 | 0.35 | 0.14 |
| i-15:0 | 0.26 | 0.22 | 0.12 | 0.18 | 0.16 | 0.23 | 0.18 | 0.25 | 0.16 | 0.24 |
| ai-15:0 | 0.13 | 0.05 | 0.00 | 0.04 | 0.04 | 0.08 | 0.04 | 0.08 | 0.05 | 0.08 |
| 15:0 | 0.28 | 0.25 | 0.20 | 0.18 | 0.19 | 0.20 | 0.19 | 0.20 | 0.23 | 0.22 |
| 15:1 | 0.20 | 0.33 | 0.19 | 0.16 | 0.19 | 0.34 | 0.16 | 0.19 | 0.13 | 0.17 |
| i-16:0 | 0.05 | 0.07 | 0.00 | 0.00 | 0.03 | 0.19 | 0.04 | 0.04 | 0.04 | 0.04 |
| ai-16:0 | 0.02 | 0.05 | 0.00 | 0.04 | 0.05 | 0.10 | 0.03 | 0.00 | 0.00 | 0.02 |
| 16:0 | 6.25 | 6.29 | 5.62 | 5.50 | 5.72 | 5.99 | 5.35 | 5.95 | 6.05 | 5.59 |
| 16:1(n-9) | 0.00 | 0.00 | 0.23 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:1(n-7) | 18.17 | 19.36 | 21.75 | 15.27 | 19.52 | 19.69 | 20.73 | 20.29 | 19.28 | 21.74 |
| 16:1(n-5) | 0.51 | 0.47 | 0.32 | 0.31 | 0.34 | 0.52 | 0.00 | 0.50 | 0.35 | 0.42 |
| i-17:0 | 0.15 | 0.09 | 0.00 | 0.03 | 0.00 | 0.11 | 0.00 | 0.06 | 0.06 | 0.07 |
| ai-17:0 | 0.43 | 0.39 | 0.29 | 0.32 | 0.29 | 0.49 | 0.43 | 0.39 | 0.30 | 0.33 |
| 16:2(n-4) | 1.19 | 1.01 | 0.96 | 0.95 | 1.00 | 0.98 | 0.97 | 0.82 | 0.92 | 1.08 |
| 16:2? | 0.26 | 0.17 | 0.00 | 0.10 | 0.10 | 0.21 | 0.07 | 0.08 | 0.06 | 0.09 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 1.96 | 1.51 | 1.62 | 1.46 | 1.47 | 1.12 | 1.40 | 1.14 | 1.45 | 1.32 |
| 17:1 | 0.00 | 0.00 | 0.04 | 0.06 | 0.10 | 0.19 | 0.00 | 0.13 | 0.08 | 0.08 |
| 16:3? | 0.43 | 0.25 | 0.27 | 0.32 | 0.40 | 0.28 | 0.28 | 0.16 | 0.20 | 0.32 |
| 16:4(n-3) | 0.66 | 0.32 | 0.34 | 0.26 | 0.23 | 0.53 | 0.43 | 0.39 | 0.28 | 0.31 |
| 16:4(n-1) | 5.68 | 5.39 | 5.11 | 5.42 | 4.55 | 4.21 | 5.05 | 4.55 | 4.53 | 4.06 |
| 18:0 | 0.50 | 0.22 | 0.17 | 0.18 | 0.30 | 0.22 | 0.18 | 0.19 | 0.22 | 0.22 |
| 18:1(n-9) | 3.19 | 2.39 | 2.40 | 1.99 | 2.49 | 1.66 | 1.71 | 1.63 | 2.38 | 2.36 |
| 18:1(n-7) | 0.92 | 0.82 | 0.74 | 0.68 | 0.80 | 0.75 | 0.69 | 0.78 | 0.83 | 0.86 |
| 18:1(n-5) | 0.45 | 0.32 | 0.29 | 0.32 | 0.40 | 0.28 | 0.31 | 0.31 | 0.32 | 0.35 |
| 18:2(n-6) | 0.55 | 0.45 | 0.42 | 0.41 | 0.41 | 0.40 | 0.37 | 0.74 | 0.45 | 0.55 |
| 18:2(n-4) | 0.10 | 0.11 | 0.03 | 0.09 | 0.16 | 0.09 | 0.10 | 0.38 | 0.08 | 0.10 |
| 18:3(n-6) | 0.25 | 0.34 | 0.42 | 0.34 | 0.59 | 0.31 | 0.37 | 0.29 | 0.30 | 0.36 |
| 18:3(n-4) | 0.03 | 0.05 | 0.00 | 0.16 | 0.05 | 0.02 | 0.03 | 0.00 | 0.03 | 0.03 |
| 18:3(n-3) | 0.20 | 0.24 | 0.18 | 0.52 | 0.27 | 0.14 | 0.13 | 0.12 | 0.15 | 0.21 |
| 18:4(n-3) | 2.03 | 2.05 | 2.33 | 2.33 | 2.07 | 2.02 | 1.71 | 1.75 | 1.96 | 2.14 |
| 18:4(n-1) | 0.33 | 0.35 | 0.31 | 0.36 | 0.36 | 0.24 | 0.35 | 0.25 | 0.28 | 0.35 |
| 20:0 | 0.05 | 0.06 | 0.06 | 0.10 | 0.04 | 0.05 | 0.04 | 0.12 | 0.08 | 0.05 |
| 20:1(n-11) | 0.00 | 0.00 | 0.00 | 0.03 | 0.05 | 0.03 | 0.02 | 0.10 | 0.05 | 0.03 |
| 20:1(n-9) | 10.36 | 10.78 | 8.83 | 9.16 | 9.15 | 5.67 | 5.87 | 5.86 | 9.27 | 8.20 |
| 20:1(n-7) | 0.22 | 0.24 | 0.16 | 0.23 | 0.29 | 0.14 | 0.14 | 0.32 | 0.19 | 0.25 |
| 20:2(n-6) | 0.03 | 0.09 | 0.00 | 0.02 | 0.13 | 0.02 | 0.05 | 0.13 | 0.03 | 0.03 |
| 20:3(n-6) | 0.05 | 0.08 | 0.07 | 0.05 | 0.09 | 0.07 | 0.09 | 0.14 | 0.09 | 0.08 |
| 20:4(n-6) | 0.36 | 0.41 | 0.42 | 0.40 | 0.54 | 0.57 | 0.51 | 0.47 | 0.53 | 0.52 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.13 | 0.00 |
| 20:4(n-3) | 0.35 | 0.35 | 0.38 | 0.34 | 0.34 | 0.36 | 0.35 | 0.36 | 0.35 | 0.42 |
| 20:5(n-3) | 23.58 | 23.84 | 25.01 | 27.98 | 22.14 | 31.89 | 32.14 | 32.54 | 28.73 | 26.74 |
| 22:0 | 0.02 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 |
| 22:1(n-11) | 5.19 | 6.46 | 4.96 | 8.24 | 4.70 | 4.68 | 3.82 | 4.50 | 5.43 | 4.68 |
| 22:1(n-9) | 1.19 | 1.21 | 0.54 | 0.59 | 0.55 | 0.52 | 0.84 | 0.53 | 0.53 | 1.07 |
| 22:1(n-7) | 0.15 | 0.14 | 0.12 | 0.12 | 0.12 | 0.09 | 0.11 | 0.08 | 0.09 | 0.13 |
| 21:5(n-3) | 0.52 | 0.38 | 0.33 | 0.98 | 1.26 | 0.30 | 0.36 | 0.32 | 0.41 | 0.31 |
| 22:5(n-3) | 0.80 | 0.65 | 1.01 | 0.92 | 1.68 | 1.10 | 0.96 | 0.87 | 0.94 | 1.05 |
| 22:6(n-3) | 2.00 | 2.02 | 4.77 | 4.72 | 6.42 | 4.43 | 4.71 | 2.96 | 2.52 | 4.12 |
| 24:1 | 0.33 | 0.39 | 0.44 | 0.24 | 1.68 | 0.30 | 0.44 | 0.32 | 0.32 | 0.33 |
| TFA (µg) | 354.1 | 325.2 | 433.3 | 398.1 | 302.0 | 300.1 | 271.5 | 213.6 | 370.4 | 414.8 |

N

Appendix 10. Continued.

| Fatty acid (%) | 54a | 54b | 58 | 66 | 76 |
|---|---|---|---|---|---|
| 14:0 | 8.79 | 8.50 | 8.40 | 8.70 | 9.72 |
| 14:1 | 0.14 | 0.16 | 0.10 | 0.13 | 0.10 |
| i-15:0 | 0.25 | 0.22 | 0.23 | 0.23 | 0.30 |
| ai-15:0 | 0.09 | 0.08 | 0.12 | 0.07 | 0.12 |
| 15:0 | 0.24 | 0.20 | 0.32 | 0.28 | 0.37 |
| 15:1 | 0.13 | 0.15 | 0.14 | 0.17 | 0.19 |
| i-16:0 | 0.05 | 0.04 | 0.04 | 0.06 | 0.07 |
| ai-16:0 | 0.02 | 0.00 | 0.00 | 0.00 | 0.04 |
| 16:0 | 6.24 | 5.94 | 6.61 | 6.13 | 7.56 |
| 16:1(n-9) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:1(n-7) | 20.03 | 18.56 | 23.79 | 18.62 | 23.75 |
| 16:1(n-5) | 0.44 | 0.34 | 0.55 | 0.35 | 0.53 |
| i-17:0 | 0.10 | 0.09 | 0.12 | 0.06 | 0.08 |
| ai-17:0 | 0.36 | 0.34 | 0.25 | 0.27 | 0.21 |
| 16:2(n-4) | 0.99 | 1.01 | 0.74 | 0.80 | 1.08 |
| 16:2? | 0.23 | 0.08 | 0.05 | 0.02 | 0.00 |
| 17:0 | 0.23 | 0.00 | 0.04 | 0.00 | 0.00 |
| 16:3(n-4) | 1.46 | 1.48 | 1.04 | 1.49 | 0.87 |
| 17:1 | 0.12 | 0.09 | 0.00 | 0.04 | 0.17 |
| 16:3? | 0.35 | 0.45 | 0.23 | 0.15 | 0.29 |
| 16:4(n-3) | 0.48 | 0.36 | 0.37 | 0.25 | 0.37 |
| 16:4(n-1) | 4.25 | 5.43 | 1.74 | 2.83 | 1.35 |
| 18:0 | 0.37 | 0.21 | 0.25 | 0.32 | 0.46 |
| 18:1(n-9) | 2.76 | 2.28 | 4.56 | 2.86 | 4.62 |
| 18:1(n-7) | 0.90 | 0.83 | 0.83 | 0.71 | 0.88 |
| 18:1(n-5) | 0.40 | 0.39 | 0.39 | 0.31 | 0.49 |
| 18:2(n-6) | 0.66 | 0.48 | 1.22 | 0.59 | 1.24 |
| 18:2(n-4) | 0.14 | 0.11 | 0.09 | 0.07 | 0.28 |
| 18:3(n-6) | 0.38 | 0.34 | 0.57 | 0.30 | 0.75 |
| 18:3(n-4) | 0.03 | 0.04 | 0.03 | 0.03 | 0.08 |
| 18:3(n-3) | 0.30 | 0.21 | 0.83 | 0.49 | 0.82 |
| 18:4(n-3) | 3.10 | 2.32 | 5.74 | 2.80 | 4.37 |
| 18:4(n-1) | 0.32 | 0.38 | 0.20 | 0.23 | 0.38 |
| 20:0 | 0.04 | 0.05 | 0.06 | 0.33 | 0.04 |
| 20:1(n-11) | 0.08 | 0.06 | 0.19 | 0.03 | 0.15 |
| 20:1(n-9) | 10.74 | 7.89 | 11.96 | 8.51 | 10.03 |
| 20:1(n-7) | 0.21 | 0.17 | 0.21 | 0.29 | 0.25 |
| 20:2(n-6) | 0.03 | 0.00 | 0.08 | 0.03 | 0.05 |
| 20:3(n-6) | 0.10 | 0.08 | 0.09 | 0.08 | 0.12 |
| 20:4(n-6) | 0.45 | 0.46 | 0.32 | 0.39 | 0.45 |
| 20:3 (n-3) | 0.04 | 0.00 | 0.06 | 0.00 | 0.02 |
| 20:4(n-3) | 0.45 | 0.36 | 0.67 | 0.43 | 0.69 |
| 20:5(n-3) | 23.22 | 28.56 | 14.09 | 20.36 | 11.01 |
| 22:0 | 0.03 | 0.00 | 0.07 | 0.07 | 0.00 |
| 22:1(n-11) | 5.51 | 4.04 | 5.34 | 6.40 | 5.31 |
| 22:1(n-9) | 0.59 | 0.51 | 0.94 | 0.58 | 0.58 |
| 22:1(n-7) | 0.11 | 0.07 | 0.16 | 0.15 | 0.12 |
| 21:5(n-3) | 0.34 | 0.41 | 0.30 | 2.04 | 0.76 |
| 22:5(n-3) | 0.83 | 1.05 | 0.60 | 1.37 | 1.00 |
| 22:6(n-3) | 2.55 | 4.86 | 4.87 | 7.62 | 6.23 |
| 24:1 | 0.32 | 0.31 | 0.39 | 1.96 | 1.67 |
| TFA (µg) | 336.2 | 247.9 | 301.8 | 329.2 | 320.8 |

Appendix 11. Complete fatty acid composition of surface-layer *Metridia longa* CVI-F (NOW; fall 1999).

| Fatty acid (%) | 14 | 45 | 66-A | 66-B | 76 |
|---|---|---|---|---|---|
| 14:0 | 2.08 | 2.24 | 1.50 | 1.37 | 2.23 |
| 14:1 | 0.16 | 0.11 | 0.13 | 0.11 | 0.45 |
| i-15:0 | 0.09 | 0.12 | 0.11 | 0.09 | 0.09 |
| ai-15:0 | 0.00 | 0.13 | 0.08 | 0.05 | 0.00 |
| 15:0 | 0.14 | 0.19 | 0.27 | 0.18 | 0.21 |
| 15:1 | 0.28 | 0.24 | 0.28 | 0.13 | 0.06 |
| i-16:0 | 0.06 | 0.07 | 0.07 | 0.07 | 0.06 |
| ai-16:0 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| 16:0 | 5.38 | 6.03 | 5.10 | 4.88 | 7.60 |
| 16:1(n-9) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:1(n-7) | 21.78 | 30.11 | 24.57 | 25.39 | 15.28 |
| 16:1(n-5) | 0.22 | 0.32 | 0.25 | 0.23 | 0.25 |
| i-17:0 | 0.09 | 0.17 | 0.15 | 0.15 | 0.15 |
| ai-17:0 | 0.13 | 0.15 | 0.09 | 0.08 | 0.14 |
| 16:2(n-4) | 0.55 | 0.62 | 0.51 | 0.44 | 0.60 |
| 16:2? | 0.76 | 1.54 | 0.99 | 0.91 | 0.59 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 0.87 | 0.54 | 0.44 | 0.37 | 0.65 |
| 17:1 | 0.00 | 0.28 | 0.31 | 0.31 | 0.00 |
| 16:3? | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-1) | 1.07 | 0.94 | 0.72 | 0.58 | 0.65 |
| 18:0 | 0.29 | 0.31 | 0.44 | 0.39 | 1.14 |
| 18:1(n-9) | 12.05 | 13.80 | 15.54 | 13.31 | 14.85 |
| 18:1(n-7) | 1.74 | 2.36 | 1.86 | 1.84 | 1.84 |
| 18:1(n-5) | 0.40 | 0.36 | 0.46 | 0.42 | 0.61 |
| 18:2(n-6) | 1.22 | 1.26 | 1.70 | 1.53 | 2.21 |
| 18:2(n-4) | 0.16 | 0.07 | 0.14 | 0.15 | 0.09 |
| 18:3(n-6) | 0.48 | 0.35 | 0.36 | 0.32 | 0.16 |
| 18:3(n-4) | 0.20 | 0.21 | 0.24 | 0.20 | 0.12 |
| 18:3(n-3) | 0.55 | 0.36 | 0.57 | 0.52 | 0.81 |
| 18:4(n-3) | 2.91 | 2.51 | 3.51 | 3.10 | 4.74 |
| 18:4(n-1) | 0.26 | 0.23 | 0.24 | 0.21 | 0.29 |
| 20:0 | 0.05 | 0.00 | 0.17 | 0.12 | 1.02 |
| 20:1(n-11) | 0.36 | 0.09 | 0.33 | 0.29 | 0.48 |
| 20:1(n-9) | 2.06 | 5.13 | 4.09 | 4.37 | 3.25 |
| 20:1(n-7) | 0.31 | 0.28 | 0.16 | 0.16 | 0.41 |
| 20:2(n-6) | 0.07 | 0.08 | 0.12 | 0.13 | 0.20 |
| 20:3(n-6) | 0.06 | 0.09 | 0.09 | 0.45 | 0.05 |
| 20:4(n-6) | 0.41 | 0.43 | 0.45 | 0.45 | 0.36 |
| 20:3 (n-3) | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-3) | 0.41 | 0.41 | 0.54 | 0.50 | 0.79 |
| 20:5(n-3) | 17.72 | 17.04 | 18.82 | 18.52 | 14.00 |
| 22:0 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 |
| 22:1(n-11) | 0.67 | 2.40 | 1.67 | 1.95 | 0.90 |
| 22:1(n-9) | 0.22 | 0.45 | 0.24 | 0.41 | 0.16 |
| 22:1(n-7) | 0.00 | 0.07 | 0.00 | 0.00 | 0.00 |
| 21:5(n-3) | 0.59 | 0.44 | 0.55 | 0.53 | 0.50 |
| 22:5(n-3) | 0.53 | 0.52 | 0.59 | 0.57 | 0.36 |
| 22:6(n-3) | 22.13 | 5.89 | 10.88 | 11.41 | 20.87 |
| 24:1 | 0.46 | 1.03 | 0.65 | 2.84 | 0.79 |
| TFA (µg) | 105.7 | 113.2 | 86.3 | 87.0 | 110.9 |

P

Appendix 12. Complete fatty acid composition of deep-layer *Metridia longa* CVI-F (NOW: fall 1999).

| Fatty acid (%) | 02 | 03-A | 03-B | 03-C | 06 | 01 | 14 | 32 | 35 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 1.97 | 1.93 | 1.43 | 1.89 | 2.29 | 2.34 | 2.26 | 1.72 | 2.28 | 1.91 |
| 14:1 | 0.11 | 0.12 | 0.10 | 0.19 | 0.20 | 0.16 | 0.15 | 0.49 | 0.12 | 0.08 |
| i-15:0 | 0.12 | 0.09 | 0.06 | 0.08 | 0.07 | 0.14 | 0.09 | 0.32 | 0.14 | 0.10 |
| ai-15:0 | 0.14 | 0.08 | 0.05 | 0.07 | 0.00 | 0.16 | 0.04 | 0.08 | 0.00 | 0.00 |
| 15:0 | 0.17 | 0.20 | 0.14 | 0.16 | 0.10 | 0.19 | 0.13 | 0.15 | 0.21 | 0.14 |
| 15:1 | 0.39 | 0.34 | 0.25 | 0.34 | 0.24 | 0.38 | 0.25 | 0.28 | 0.28 | 0.21 |
| i-16:0 | 0.00 | 0.05 | 0.00 | 0.05 | 0.00 | 0.07 | 0.05 | 0.12 | 0.00 | 0.06 |
| ai-16:0 | 0.00 | 0.06 | 0.05 | 0.00 | 0.00 | 0.03 | 0.06 | 0.15 | 0.00 | 0.00 |
| 16:0 | 5.60 | 5.50 | 4.73 | 4.92 | 4.70 | 6.03 | 4.77 | 5.21 | 5.96 | 4.85 |
| 16:1(n-9) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.02 |
| 16:1(n-7) | 31.61 | 32.32 | 30.27 | 31.65 | 23.47 | 30.61 | 22.68 | 22.17 | 35.35 | 31.02 |
| 16:1(n-5) | 0.35 | 0.32 | 0.30 | 0.28 | 0.22 | 0.33 | 0.19 | 0.20 | 0.00 | 0.22 |
| i-17:0 | 0.18 | 0.17 | 0.12 | 0.13 | 0.09 | 0.19 | 0.11 | 0.05 | 0.19 | 0.12 |
| ai-17:0 | 0.17 | 0.19 | 0.15 | 0.16 | 0.12 | 0.21 | 0.15 | 0.15 | 0.23 | 0.14 |
| 16:2(n-4) | 0.63 | 0.58 | 0.47 | 0.55 | 0.54 | 0.64 | 0.52 | 0.48 | 0.65 | 0.54 |
| 16:2? | 1.48 | 1.43 | 1.29 | 1.29 | 1.08 | 1.06 | 0.59 | 0.80 | 0.94 | 1.15 |
| 17:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3(n-4) | 0.91 | 0.87 | 0.79 | 0.86 | 0.70 | 0.60 | 0.72 | 0.52 | 0.57 | 0.70 |
| 17:1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.34 | 0.00 | 0.00 | 0.35 | 0.00 |
| 16:3? | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-1) | 1.01 | 0.86 | 0.78 | 0.83 | 0.88 | 1.09 | 0.90 | 0.93 | 0.97 | 0.86 |
| 18:0 | 0.41 | 0.36 | 0.30 | 0.33 | 0.26 | 0.50 | 0.35 | 0.46 | 0.50 | 0.31 |
| 18:1(n-9) | 13.45 | 13.46 | 10.65 | 12.94 | 12.01 | 14.91 | 11.44 | 11.65 | 13.68 | 14.27 |
| 18:1(n-7) | 2.41 | 2.50 | 2.57 | 2.30 | 1.76 | 2.58 | 1.77 | 1.96 | 2.40 | 1.74 |
| 18:1(n-5) | 0.40 | 0.39 | 0.36 | 0.35 | 0.32 | 0.50 | 0.38 | 0.29 | 0.42 | 0.27 |
| 18:2(n-6) | 1.29 | 1.24 | 1.09 | 1.28 | 1.09 | 1.35 | 1.06 | 1.19 | 1.31 | 1.38 |
| 18:2(n-4) | 0.16 | 0.19 | 0.18 | 0.19 | 0.00 | 0.32 | 0.09 | 0.13 | 0.17 | 0.09 |
| 18:3(n-6) | 0.56 | 0.48 | 0.52 | 0.60 | 0.29 | 0.56 | 0.45 | 0.27 | 0.44 | 0.43 |
| 18:3(n-4) | 0.27 | 0.26 | 0.22 | 0.23 | 0.16 | 0.33 | 0.30 | 0.87 | 0.26 | 0.19 |
| 18:3(n-3) | 0.67 | 0.56 | 0.53 | 0.66 | 0.55 | 0.57 | 0.70 | 0.32 | 0.39 | 0.36 |
| 18:4(n-3) | 2.89 | 2.36 | 2.45 | 2.65 | 2.24 | 2.67 | 2.37 | 2.05 | 2.81 | 2.59 |
| 18:4(n-1) | 0.28 | 0.25 | 0.21 | 0.21 | 0.23 | 0.27 | 0.21 | 0.21 | 0.30 | 0.27 |
| 20:0 | 0.00 | 0.04 | 0.00 | 0.00 | 0.05 | 0.00 | 0.06 | 0.11 | 0.00 | 0.00 |
| 20:1(n-11) | 0.00 | 0.08 | 0.06 | 0.08 | 0.11 | 0.09 | 0.08 | 0.07 | 0.00 | 0.00 |
| 20:1(n-9) | 4.66 | 4.05 | 2.61 | 3.85 | 3.90 | 4.64 | 2.95 | 3.71 | 5.24 | 5.99 |
| 20:1(n-7) | 0.32 | 0.28 | 0.24 | 0.25 | 0.17 | 0.27 | 0.25 | 0.94 | 0.36 | 0.33 |
| 20:2(n-6) | 0.00 | 0.10 | 0.09 | 0.09 | 0.09 | 0.10 | 0.40 | 1.40 | 0.19 | 0.14 |
| 20:3(n-6) | 0.00 | 0.10 | 0.29 | 0.11 | 0.00 | 0.07 | 1.29 | 0.18 | 0.00 | 0.09 |
| 20:4(n-6) | 0.62 | 0.58 | 1.02 | 0.59 | 0.43 | 0.55 | 0.46 | 0.80 | 0.37 | 0.00 |
| 20:3 (n-3) | 0.00 | 0.05 | 0.00 | 0.05 | 0.00 | 0.06 | 0.00 | 0.06 | 0.00 | 0.00 |
| 20:4(n-3) | 0.46 | 0.45 | 0.65 | 0.51 | 0.39 | 0.42 | 0.40 | 0.87 | 0.44 | 0.44 |
| 20:5(n-3) | 16.46 | 16.76 | 17.36 | 18.71 | 18.00 | 16.56 | 16.43 | 18.58 | 14.27 | 16.64 |
| 22:0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 |
| 22:1(n-11) | 2.14 | 1.66 | 0.81 | 1.53 | 1.91 | 2.34 | 1.22 | 2.15 | 2.24 | 3.15 |
| 22:1(n-9) | 0.59 | 0.30 | 0.20 | 0.30 | 0.26 | 0.18 | 0.22 | 0.31 | 0.62 | 0.44 |
| 22:1(n-7) | 0.12 | 0.06 | 0.06 | 0.07 | 0.04 | 0.06 | 0.03 | 0.04 | 0.00 | 0.33 |
| 21:5(n-3) | 0.60 | 0.47 | 0.53 | 0.52 | 2.28 | 0.57 | 1.11 | 0.45 | 0.35 | 2.83 |
| 22:5(n-3) | 0.60 | 0.46 | 2.85 | 0.51 | 2.76 | 0.49 | 0.95 | 1.82 | 0.41 | 0.00 |
| 22:6(n-3) | 4.65 | 6.46 | 10.21 | 6.74 | 14.11 | 3.73 | 17.41 | 13.35 | 3.52 | 4.58 |
| 24:1 | 1.17 | 0.94 | 2.94 | 0.90 | 1.88 | 0.77 | 3.93 | 1.92 | 1.06 | 1.00 |
| TFA (µg) | 96.4 | 88.0 | 86.4 | 103.0 | 137.0 | 86.9 | 127.8 | 167.6 | 96.7 | 51.0 |

Q

Appendix 12. Continued.

| Fatty acid (%) | 45 | 54a-B | 54a-C | 54b | 58 | 66 | 76 |
|---|---|---|---|---|---|---|---|
| 14:0 | 2.77 | 1.69 | 1.40 | 2.05 | 2.71 | 1.11 | 1.77 |
| 14:1 | 0.12 | 0.09 | 0.06 | 0.12 | 0.00 | 2.07 | 0.92 |
| i-15:0 | 0.14 | 0.00 | 0.07 | 0.11 | 0.17 | 0.00 | 0.00 |
| ai-15:0 | 0.00 | 0.14 | 0.07 | 0.06 | 0.36 | 0.00 | 0.00 |
| 15:0 | 0.19 | 0.23 | 0.17 | 0.15 | 1.11 | 0.05 | 0.15 |
| 15:1 | 0.33 | 0.27 | 0.27 | 0.27 | 1.01 | 0.02 | 0.07 |
| i-16:0 | 0.00 | 0.14 | 0.26 | 0.07 | 0.82 | 0.00 | 0.00 |
| ai-16:0 | 0.00 | 0.00 | 0.15 | 0.07 | 0.14 | 0.20 | 0.16 |
| 16:0 | 6.24 | 6.33 | 4.95 | 5.56 | 13.05 | 4.64 | 6.61 |
| 16:1(n-9) | 0.00 | 0.00 | 0.05 | 0.11 | 0.00 | 0.10 | 0.00 |
| 16:1(n-7) | 31.09 | 28.82 | 18.15 | 27.69 | 19.14 | 16.03 | 14.01 |
| 16:1(n-5) | 0.34 | 0.26 | 1.16 | 0.28 | 0.84 | 0.14 | 0.20 |
| i-17:0 | 0.16 | 0.16 | 0.17 | 0.12 | 0.11 | 0.38 | 0.11 |
| ai-17:0 | 0.15 | 0.18 | 0.15 | 0.14 | 0.00 | 0.47 | 0.00 |
| 16:2(n-4) | 0.66 | 0.46 | 0.46 | 0.58 | 0.55 | 0.29 | 1.21 |
| 16:2? | 0.88 | 1.57 | 1.02 | 1.33 | 1.24 | 0.66 | 0.60 |
| 17:0 | 0.00 | 0.08 | 0.00 | 0.00 | 0.19 | 0.00 | 0.00 |
| 16:3(n-4) | 0.72 | 0.61 | 0.70 | 0.63 | 0.76 | 0.12 | 0.28 |
| 17:1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:3? | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-1) | 1.18 | 0.58 | 0.66 | 1.05 | 0.27 | 0.31 | 0.84 |
| 18:0 | 0.34 | 0.38 | 0.33 | 0.38 | 1.12 | 0.70 | 1.30 |
| 18:1(n-9) | 14.25 | 15.28 | 10.79 | 13.98 | 12.65 | 10.66 | 16.44 |
| 18:1(n-7) | 2.36 | 2.13 | 1.33 | 2.17 | 3.24 | 1.44 | 1.74 |
| 18:1(n-5) | 0.46 | 0.57 | 1.16 | 0.45 | 1.27 | 1.08 | 0.69 |
| 18:2(n-6) | 1.05 | 1.67 | 1.00 | 1.42 | 2.16 | 4.91 | 2.28 |
| 18:2(n-4) | 0.32 | 0.09 | 0.10 | 0.16 | 0.13 | 0.00 | 0.00 |
| 18:3(n-6) | 2.37 | 0.33 | 0.24 | 0.33 | 0.22 | 0.44 | 1.31 |
| 18:3(n-4) | 0.75 | 0.19 | 0.25 | 0.16 | 0.14 | 0.36 | 0.08 |
| 18:3(n-3) | 0.36 | 0.51 | 0.30 | 0.47 | 0.47 | 0.79 | 1.00 |
| 18:4(n-3) | 2.50 | 2.60 | 2.56 | 2.90 | 1.56 | 3.11 | 4.32 |
| 18:4(n-1) | 0.24 | 0.57 | 2.70 | 0.26 | 0.15 | 0.00 | 0.23 |
| 20:0 | 0.53 | 1.05 | 0.00 | 0.16 | 0.37 | 0.40 | 0.72 |
| 20:1(n-11) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.30 |
| 20:1(n-9) | 2.06 | 2.78 | 8.67 | 1.34 | 7.23 | 2.36 | 4.57 |
| 20:1(n-7) | 0.00 | 0.00 | 4.82 | 0.00 | 0.24 | 1.02 | 0.00 |
| 20:2(n-6) | 0.18 | 0.13 | 1.48 | 0.15 | 0.14 | 0.06 | 0.06 |
| 20:3(n-6) | 0.50 | 0.08 | 0.07 | 0.09 | 0.00 | 0.02 | 0.00 |
| 20:4(n-6) | 2.59 | 0.97 | 0.31 | 0.85 | 0.19 | 0.25 | 0.33 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.00 | 0.86 | 0.00 | 0.00 | 0.00 |
| 20:4(n-3) | 0.40 | 0.47 | 9.01 | 0.44 | 0.00 | 0.41 | 0.50 |
| 20:5(n-3) | 15.37 | 14.74 | 14.52 | 19.71 | 11.05 | 13.73 | 8.11 |
| 22:0 | 0.00 | 0.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.14 |
| 22:1(n-11) | 2.18 | 2.30 | 2.50 | 1.52 | 2.90 | 0.81 | 1.47 |
| 22:1(n-9) | 0.63 | 0.51 | 0.58 | 0.55 | 0.38 | 0.00 | 0.24 |
| 22:1(n-7) | 0.12 | 0.00 | 0.00 | 0.06 | 0.00 | 0.00 | 0.00 |
| 21:5(n-3) | 0.00 | 0.87 | 0.32 | 0.00 | 0.00 | 0.00 | 1.01 |
| 22:5(n-3) | 0.46 | 0.92 | 0.49 | 0.71 | 0.21 | 4.41 | 1.59 |
| 22:6(n-3) | 4.35 | 8.39 | 5.95 | 9.78 | 9.77 | 22.15 | 16.14 |
| 24:1 | 0.69 | 0.85 | 0.59 | 0.64 | 1.92 | 4.29 | 8.51 |
| TFA (µg) | 61.1 | 32.5 | 37.7 | 67.0 | 16.0 | 128.6 | 35.4 |

R

Appendix 13. Complete fatty acid composition of seston collected at 1% light (NOW; fall 1999).

| Fatty acid (%) | 03-A | 03-B | 03-C | 06 | 01 | 14 | 32-A | 32-B | 32-C | 35 | 40 | 45 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 13.12 | 12.33 | 12.43 | 10.57 | 12.13 | 13.50 | 10.57 | 10.72 | 10.65 | 10.74 | 11.87 | 11.17 |
| 14:1 | 0.13 | 0.16 | 0.19 | 0.13 | 0.19 | 0.00 | 0.31 | 0.45 | 0.43 | 0.29 | 0.28 | 0.16 |
| i-15:0 | 0.33 | 0.33 | 0.38 | 0.92 | 0.71 | 1.37 | 0.70 | 0.61 | 0.66 | 0.71 | 0.81 | 0.78 |
| ai-15:0 | 0.29 | 0.45 | 0.49 | 0.61 | 0.36 | 0.62 | 0.81 | 0.27 | 0.55 | 0.74 | 1.21 | 0.69 |
| 15:0 | 0.46 | 0.45 | 0.57 | 0.47 | 0.45 | 1.02 | 0.58 | 0.48 | 0.61 | 0.49 | 0.48 | 0.51 |
| 15:1 | 0.05 | 0.07 | 0.09 | 0.43 | 0.17 | 0.75 | 0.32 | 0.17 | 0.27 | 0.30 | 0.34 | 0.28 |
| i-16:0 | 0.07 | 0.07 | 0.08 | 0.45 | 0.12 | 0.51 | 0.21 | 0.19 | 0.18 | 0.17 | 1.11 | 0.66 |
| ai-16:0 | 0.33 | 0.03 | 0.32 | 0.89 | 0.09 | 0.36 | 0.55 | 0.45 | 0.23 | 0.14 | 1.16 | 0.57 |
| 16:0 | 15.18 | 14.41 | 19.05 | 12.33 | 11.72 | 11.36 | 12.96 | 13.25 | 13.72 | 13.47 | 13.09 | 16.62 |
| 16:1(n-9) | 0.63 | 0.48 | 0.52 | 1.43 | 1.80 | 0.42 | 0.91 | 0.51 | 0.00 | 0.96 | 2.34 | 1.14 |
| 16:1(n-7) | 34.45 | 31.90 | 33.26 | 20.61 | 25.26 | 23.75 | 23.89 | 25.41 | 25.12 | 29.43 | 15.53 | 26.17 |
| 16:1(n-5) | 0.90 | 0.78 | 1.20 | 0.69 | 0.65 | 0.28 | 0.63 | 0.63 | 0.65 | 0.93 | 0.71 | 0.66 |
| i-17:0 | 0.55 | 0.51 | 0.52 | 1.02 | 1.06 | 0.78 | 0.67 | 0.50 | 0.50 | 0.74 | 0.77 | 0.67 |
| ai-17:0 | 0.44 | 0.40 | 0.38 | 0.53 | 0.53 | 0.32 | 0.40 | 0.35 | 0.32 | 0.43 | 0.26 | 0.40 |
| 16:2(n-4) | 1.41 | 1.33 | 1.19 | 1.82 | 1.99 | 1.26 | 1.28 | 1.27 | 0.96 | 1.48 | 1.34 | 1.26 |
| 16:2? | 0.09 | 0.05 | 0.11 | 0.00 | 0.11 | 0.00 | 0.42 | 0.27 | 0.28 | 0.19 | 0.47 | 0.20 |
| 17:0 | 0.06 | 0.06 | 0.12 | 0.15 | 0.08 | 0.00 | 0.18 | 0.00 | 0.00 | 0.12 | 0.18 | 0.16 |
| 16:3(n-4) | 0.79 | 0.69 | 0.64 | 1.31 | 1.46 | 1.04 | 1.00 | 0.94 | 0.89 | 1.05 | 1.68 | 0.66 |
| 17:1 | 0.12 | 0.17 | 0.10 | 0.10 | 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.21 | 0.13 | 0.00 |
| 16:3? | 0.64 | 0.63 | 0.58 | 1.12 | 1.31 | 0.80 | 0.47 | 0.36 | 0.35 | 0.95 | 0.89 | 0.95 |
| 16:4(n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-1) | 2.64 | 2.86 | 2.08 | 4.91 | 5.65 | 3.78 | 2.32 | 2.41 | 1.93 | 3.56 | 3.48 | 3.19 |
| 18:0 | 0.64 | 0.67 | 1.01 | 1.11 | 0.78 | 1.16 | 1.02 | 1.02 | 1.06 | 0.75 | 1.23 | 0.95 |
| 18:1(n-9) | 1.87 | 1.68 | 2.50 | 2.16 | 1.42 | 3.82 | 3.30 | 4.87 | 5.19 | 2.85 | 5.05 | 6.37 |
| 18:1(n-7) | 0.96 | 0.90 | 1.10 | 1.60 | 1.24 | 0.78 | 1.99 | 2.16 | 2.04 | 1.33 | 2.07 | 1.80 |
| 18:1(n-5) | 0.14 | 0.14 | 0.20 | 0.23 | 0.17 | 0.00 | 0.30 | 0.34 | 0.32 | 0.21 | 0.36 | 0.23 |
| 18:2(n-6) | 0.99 | 0.94 | 1.15 | 0.83 | 0.75 | 0.80 | 0.93 | 1.01 | 0.91 | 0.97 | 1.81 | 0.91 |
| 18:2(n-4) | 0.25 | 0.09 | 0.16 | 0.41 | 0.20 | 0.35 | 1.56 | 0.41 | 0.97 | 0.14 | 1.30 | 0.14 |
| 18:3(n-6) | 0.40 | 0.36 | 0.44 | 0.29 | 0.34 | 0.32 | 0.00 | 0.31 | 0.23 | 0.35 | 0.23 | 0.32 |
| 18:3(n-4) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 18:3(n-3) | 0.27 | 0.26 | 0.30 | 0.24 | 0.27 | 0.34 | 0.21 | 0.17 | 0.31 | 0.29 | 0.31 | 0.23 |
| 18:4(n-3) | 1.68 | 1.71 | 1.74 | 1.47 | 1.73 | 1.04 | 1.17 | 1.45 | 1.21 | 2.27 | 1.45 | 1.49 |
| 18:4(n-1) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:0 | 0.08 | 0.06 | 0.09 | 0.12 | 0.00 | 0.10 | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 | 0.00 |
| 20:1(n-11) | 0.34 | 0.28 | 0.38 | 0.45 | 0.53 | 1.00 | 0.50 | 0.42 | 0.36 | 0.49 | 0.69 | 0.32 |
| 20:1(n-9) | 0.12 | 0.06 | 0.05 | 0.38 | 0.19 | 0.30 | 0.00 | 0.92 | 0.64 | 0.94 | 0.44 | 0.00 |
| 20:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:2(n-6) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:3(n-6) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-6) | 0.43 | 0.47 | 0.33 | 0.55 | 0.30 | 0.47 | 0.33 | 0.29 | 0.37 | 0.34 | 0.26 | 0.29 |
| 20:3(n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-3) | 0.48 | 0.44 | 0.32 | 0.23 | 0.33 | 0.26 | 0.21 | 0.26 | 0.31 | 0.33 | 0.26 | 0.27 |
| 20:5(n-3) | 14.35 | 20.04 | 11.22 | 16.25 | 19.72 | 18.49 | 19.33 | 19.22 | 18.79 | 15.66 | 15.87 | 14.84 |
| 22:0 | 0.05 | 0.08 | 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.07 | 0.00 | 0.00 |
| 22:1(n-11) | 0.15 | 0.12 | 0.12 | 0.41 | 0.17 | 1.23 | 0.00 | 0.81 | 0.64 | 0.83 | 0.37 | 0.22 |
| 22:1(n-9) | 0.48 | 0.58 | 0.66 | 0.45 | 0.22 | 1.43 | 0.25 | 0.32 | 0.28 | 0.36 | 0.86 | 0.27 |
| 22:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.25 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 21:5(n-3) | 0.40 | 0.09 | 0.22 | 0.00 | 0.18 | 0.21 | 1.92 | 0.36 | 1.10 | 0.24 | 1.33 | 0.14 |
| 22:5(n-3) | 0.21 | 0.22 | 0.22 | 3.36 | 0.35 | 0.65 | 1.51 | 0.35 | 0.46 | 0.29 | 0.80 | 0.25 |
| 22:6(n-3) | 2.92 | 2.53 | 3.27 | 7.10 | 4.97 | 4.26 | 5.84 | 6.06 | 6.00 | 3.95 | 7.02 | 4.07 |
| 24:1 | 0.11 | 0.12 | 0.13 | 1.89 | 0.17 | 1.06 | 0.20 | 0.00 | 0.50 | 0.14 | 0.17 | 0.00 |
| TFA (ng $ml^{-1}$) | 53.5 | 60.3 | 48.2 | 26.0 | 39.7 | 25.8 | 13.2 | 16.7 | 15.5 | 40.8 | 18.6 | 25.4 |
| TL (ng $ml^{-1}$) | 78.7 | 87.5 | 66.9 | 57.1 | 74.1 | 50.2 | 21.0 | 26.1 | 24.7 | 65.7 | 40.3 | 33.0 |

Appendix 13. Continued.

| Fatty acid (%) | 50a | 50b-A | 50b-B | 50b-C | 54a | 54b | 58-A | 58-B | 58-C | 66 | 68 | 76 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 9.66 | 11.22 | 11.44 | 12.03 | 6.41 | 9.89 | 11.94 | 9.80 | 10.56 | 9.69 | 7.95 | 11.81 |
| 14:1 | 1.46 | 0.14 | 0.24 | 0.26 | 0.76 | 0.39 | 0.19 | 0.50 | 0.29 | 0.91 | 0.53 | 0.47 |
| i-15:0 | 0.45 | 1.04 | 1.00 | 1.18 | 0.88 | 1.07 | 0.74 | 0.76 | 0.92 | 0.99 | 0.66 | 1.32 |
| ai-15:0 | 0.66 | 1.01 | 1.05 | 0.83 | 0.71 | 1.06 | 0.89 | 1.17 | 1.20 | 0.64 | 0.77 | 0.55 |
| 15:0 | 0.56 | 0.51 | 0.48 | 0.52 | 0.58 | 0.56 | 0.53 | 0.62 | 0.70 | 0.77 | 0.48 | 0.68 |
| 15:1 | 0.32 | 0.22 | 0.16 | 0.27 | 0.32 | 0.24 | 0.16 | 0.13 | 0.19 | 0.67 | 0.34 | 0.36 |
| i-16:0 | 0.24 | 0.18 | 0.22 | 0.18 | 1.08 | 1.07 | 0.33 | 0.30 | 0.46 | 0.77 | 0.77 | 0.69 |
| ai-16:0 | 0.54 | 0.12 | 0.10 | 0.32 | 0.82 | 1.15 | 0.14 | 1.49 | 0.21 | 0.39 | 1.29 | 0.46 |
| 16:0 | 8.83 | 14.30 | 13.40 | 13.97 | 10.04 | 25.00 | 11.63 | 13.39 | 14.06 | 10.76 | 15.55 | 12.70 |
| 16:1(n-9) | 0.00 | 2.10 | 2.16 | 0.73 | 0.82 | 1.96 | 1.78 | 2.63 | 2.50 | 0.67 | 1.36 | 0.67 |
| 16:1(n-7) | 21.93 | 24.60 | 23.34 | 25.58 | 14.08 | 13.73 | 18.46 | 16.62 | 17.45 | 15.51 | 11.82 | 11.40 |
| 16:1(n-5) | 0.56 | 0.57 | 0.58 | 0.58 | 0.62 | 0.87 | 0.62 | 0.83 | 0.87 | 0.87 | 1.26 | 0.64 |
| i-17:0 | 0.50 | 0.83 | 0.85 | 0.80 | 0.77 | 1.13 | 0.85 | 1.03 | 1.14 | 0.93 | 0.89 | 0.90 |
| ai-17:0 | 0.31 | 0.24 | 0.44 | 0.27 | 0.88 | 0.33 | 0.66 | 0.31 | 0.24 | 1.02 | 0.94 | 0.25 |
| 16:2(n-4) | 1.03 | 1.79 | 2.09 | 1.82 | 1.81 | 1.42 | 1.68 | 1.91 | 1.62 | 1.36 | 1.39 | 1.38 |
| 16:2? | 0.49 | 0.23 | 0.17 | 0.19 | 1.22 | 0.33 | 0.29 | 0.62 | 0.41 | 0.21 | 0.90 | 0.00 |
| 17:0 | 0.33 | 0.10 | 0.13 | 0.09 | 0.94 | 0.22 | 0.15 | 0.68 | 0.34 | 0.34 | 0.82 | 0.00 |
| 16:3(n-4) | 1.91 | 2.06 | 2.18 | 2.03 | 3.01 | 1.71 | 2.54 | 2.35 | 2.00 | 2.95 | 2.44 | 1.46 |
| 17:1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.42 | 0.21 | 0.00 | 0.00 | 0.00 |
| 16:3? | 0.54 | 0.77 | 0.84 | 0.75 | 0.82 | 0.81 | 0.84 | 1.28 | 1.13 | 0.36 | 1.07 | 0.88 |
| 16:4(n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-1) | 3.14 | 3.10 | 3.23 | 3.01 | 2.31 | 2.49 | 3.81 | 3.24 | 3.27 | 2.63 | 2.86 | 2.91 |
| 18:0 | 1.12 | 0.94 | 1.04 | 0.94 | 2.26 | 1.74 | 0.72 | 1 28 | 1.36 | 2.88 | 2.58 | 1.57 |
| 18:1(n-9) | 6.30 | 2.28 | 2.38 | 2.42 | 10.15 | 3.11 | 3.39 | 3.50 | 2.89 | 5.25 | 6.41 | 5.18 |
| 18:1(n-7) | 3.08 | 1.65 | 1.68 | 1.62 | 2.54 | 2.02 | 1.38 | 1.93 | 1.70 | 1.47 | 2.37 | 1.04 |
| 18:1(n-5) | 0.28 | 0.27 | 0.31 | 0.23 | 0.53 | 0.37 | 0.26 | 0.35 | 0.29 | 0.52 | 1.00 | 0.00 |
| 18:2(n-6) | 0.79 | 1.21 | 1.14 | 1.23 | 1.58 | 0.87 | 1.10 | 1.25 | 1.23 | 2.60 | 1.77 | 2.42 |
| 18:2(n-4) | 0.00 | 0.73 | 0.30 | 0.33 | 0.65 | 0.53 | 0.34 | 0.39 | 0.47 | 1.81 | 1.53 | 0.50 |
| 18:3(n-6) | 0.23 | 0.32 | 0.36 | 0.44 | 0.28 | 0.23 | 0.24 | 0.17 | 0.18 | 0.68 | 0.27 | 0.42 |
| 18:3(n-4) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.45 | 0.00 | 0.17 | 0.00 | 0.00 | 0.35 | 0.00 |
| 18:3(n-3) | 0.25 | 0.44 | 0.71 | 0.42 | 1.90 | 0.37 | 0.65 | 0.58 | 1.40 | 0.86 | 0.92 | 1.47 |
| 18:4(n-3) | 1.93 | 2.13 | 2.06 | 2.24 | 5.46 | 1.39 | 3.44 | 2.96 | 3.24 | 2.84 | 2.99 | 3.83 |
| 18:4(n-1) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:0 | 0.29 | 0.00 | 0.12 | 0.08 | 0.00 | 0.19 | 0.00 | 0.13 | 0.26 | 0.00 | 0.70 | 0.43 |
| 20:1(n-11) | 0.39 | 0.73 | 0.72 | 0.66 | 1.71 | 1.25 | 2.38 | 2.36 | 2.76 | 2.52 | 2.13 | 4.63 |
| 20:1(n-9) | 3.82 | 0.09 | 0.34 | 0.35 | 0.68 | 0.24 | 0.29 | 0.71 | 0.72 | 0.91 | 1.09 | 0.60 |
| 20:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:2(n-6) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:3(n-6) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-6) | 0.46 | 0.46 | 0.47 | 0.47 | 0.32 | 0.31 | 0.25 | 0.17 | 0.22 | 1.10 | 0.00 | 0.34 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-3) | 0.31 | 0.28 | 0.31 | 0.28 | 0.56 | 0.38 | 0.40 | 0.47 | 0.40 | 0.39 | 0.34 | 0.56 |
| 20:5(n-3) | 19.27 | 16.14 | 16.74 | 16.19 | 12.45 | 10.34 | 17.69 | 13.03 | 13.73 | 13.87 | 10.68 | 10.29 |
| 22:0 | 0.00 | 0.08 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.14 | 0.00 | 0.00 | 0.00 | 0.00 |
| 22:1(n-11) | 2.83 | 0.09 | 0.21 | 0.26 | 0.00 | 0.36 | 0.12 | 0.40 | 0.29 | 0.97 | 0.22 | 0.42 |
| 22:1(n-9) | 0.23 | 0.13 | 0.26 | 0.25 | 0.00 | 0.00 | 0.68 | 2.79 | 1.61 | 0.56 | 0.19 | 0.00 |
| 22:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 21:5(n-3) | 0.29 | 0.80 | 0.47 | 0.43 | 0.57 | 0.91 | 0.36 | 0.29 | 0.41 | 0.28 | 1.16 | 0.48 |
| 22:5(n-3) | 1.19 | 0.11 | 0.14 | 0.14 | 0.67 | 1.37 | 0.28 | 0.38 | 0.55 | 0.95 | 9.01 | 0.00 |
| 22:6(n-3) | 3.49 | 5.83 | 5.88 | 5.51 | 8.81 | 6.71 | 7.65 | 6.31 | 6.39 | 6.66 | 0.58 | 16.30 |
| 24:1 | 0.00 | 0.15 | 0.19 | 0.12 | 0.00 | 0.29 | 0.16 | 0.17 | 0.13 | 0.43 | 8.46 | 0.00 |
| TFA (ng $ml^{-1}$) | 13.4 | 28.5 | 30.2 | 27.1 | 10.1 | 17.4 | 25.1 | 23.0 | 22.8 | 16.6 | 20.4 | 17.0 |
| TL (ng $ml^{-1}$) | 23.6 | 50.4 | 70.3 | 56.4 | 22.0 | 40.9 | 46.0 | 53.3 | 50.4 | 42.1 | 30.8 | 37.4 |

T

Appendix 14. Complete fatty acid composition of seston collected below 1% light (NOW; fall 1999).

| Fatty acid (%) | 02 | 03 | 06 | 01 | 14 | 32 | 35 | 40 | 45 | 54a |
|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 12.68 | 8.59 | 9.14 | 9.60 | 8.63 | 14.02 | 12.96 | 12.83 | 9.02 | 11.08 |
| 14:1 | 0.18 | 1.94 | 0.96 | 0.74 | 0.98 | 0.15 | 0.61 | 0.00 | 0.19 | 0.00 |
| i-15:0 | 2.03 | 0.44 | 0.86 | 0.40 | 1.02 | 1.23 | 0.23 | 2.02 | 1.61 | 2.31 |
| ai-15:0 | 0.40 | 11.76 | 0.45 | 0.57 | 0.61 | 1.06 | 0.58 | 0.30 | 0.26 | 0.56 |
| 15:0 | 1.33 | 0.82 | 0.83 | 0.69 | 1.40 | 1.06 | 0.74 | 0.58 | 0.75 | 0.71 |
| 15:1 | 1.05 | 1.35 | 0.62 | 0.38 | 0.68 | 0.81 | 0.59 | 0.12 | 0.16 | 0.61 |
| i-16:0 | 0.68 | 0.27 | 0.77 | 0.27 | 0.28 | 0.35 | 0.31 | 0.12 | 0.31 | 0.37 |
| ai-16:0 | 0.46 | 0.00 | 0.61 | 0.42 | 0.46 | 0.40 | 0.23 | 0.00 | 0.14 | 0.00 |
| 16:0 | 18.26 | 12.17 | 14.98 | 13.48 | 17.55 | 18.66 | 17.61 | 21.43 | 15.05 | 18.30 |
| 16:1(n-9) | 0.42 | 0.00 | 0.89 | 0.71 | 0.34 | 0.30 | 0.00 | 0.00 | 0.16 | 0.00 |
| 16:1(n-7) | 23.69 | 18.73 | 24.17 | 25.22 | 18.07 | 27.56 | 32.71 | 33.76 | 22.56 | 27.50 |
| 16:1(n-5) | 0.39 | 8.71 | 0.55 | 0.54 | 0.61 | 1.54 | 0.39 | 0.40 | 1.00 | 0.47 |
| i-17:0 | 0.00 | 0.94 | 0.59 | 0.48 | 0.91 | 1.04 | 0.51 | 0.11 | 0.35 | 0.35 |
| ai-17:0 | 0.21 | 0.39 | 0.34 | 0.44 | 0.34 | 0.39 | 0.00 | 0.00 | 0.22 | 0.00 |
| 16:2(n-4) | 0.63 | 1.02 | 1.23 | 1.10 | 1.27 | 1.09 | 0.63 | 0.66 | 0.67 | 0.62 |
| 16:2? | 1.10 | 0.00 | 0.12 | 0.78 | 0.14 | 0.00 | 0.24 | 0.43 | 0.44 | 0.63 |
| 17:0 | 0.51 | 0.58 | 0.55 | 0.33 | 0.47 | 0.46 | 0.17 | 0.10 | 0.22 | 0.23 |
| 16:3(n-4) | 0.74 | 0.77 | 0.82 | 1.21 | 1.01 | 0.85 | 0.74 | 0.20 | 0.40 | 0.29 |
| 17:1 | 0.00 | 0.00 | 0.10 | 0.00 | 0.00 | 0.15 | 0.00 | 0.00 | 0.45 | 0.00 |
| 16:3? | 0.60 | 1.28 | 0.36 | 0.29 | 0.69 | 0.21 | 0.16 | 0.13 | 0.13 | 0.32 |
| 16:4(n-3) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-1) | 1.35 | 0.75 | 1.63 | 2.19 | 2.22 | 1.94 | 1.42 | 0.47 | 0.89 | 0.56 |
| 18:0 | 4.78 | 7.60 | 6.13 | 3.29 | 6.02 | 2.20 | 2.50 | 2.34 | 2.15 | 3.42 |
| 18:1(n-9) | 5.24 | 7.86 | 3.87 | 8.10 | 6.68 | 7.00 | 5.71 | 2.31 | 17.82 | 6.59 |
| 18:1(n-7) | 1.24 | 0.00 | 1.36 | 1.72 | 1.18 | 1.70 | 1.41 | 0.68 | 2.02 | 1.47 |
| 18:1(n-5) | 0.00 | 5.54 | 0.18 | 0.00 | 0.29 | 0.33 | 0.18 | 0.00 | 0.37 | 0.25 |
| 18:2(n-6) | 1.40 | 0.00 | 1.58 | 1.21 | 3.02 | 2.67 | 2.81 | 4.37 | 2.22 | 2.51 |
| 18:2(n-4) | 2.58 | 1.26 | 1.43 | 1.40 | 1.21 | 0.67 | 0.34 | 2.39 | 0.39 | 1.17 |
| 18:3(n-6) | 0.25 | 0.29 | 0.34 | 0.42 | 0.17 | 0.15 | 0.26 | 0.44 | 0.29 | 0.38 |
| 18:3(n-4) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.16 | 0.11 | 0.00 |
| 18:3(n-3) | 0.60 | 0.99 | 0.47 | 0.44 | 0.90 | 0.19 | 0.26 | 0.34 | 0.38 | 0.43 |
| 18:4(n-3) | 1.35 | 1.33 | 0.85 | 1.45 | 1.25 | 0.99 | 0.94 | 2.08 | 1.17 | 0.86 |
| 18:4(n-1) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:0 | 0.47 | 0.00 | 0.00 | 0.40 | 0.40 | 0.00 | 0.00 | 0.23 | 0.24 | 0.36 |
| 20:1(n-11) | 0.58 | 0.00 | 0.28 | 0.65 | 0.65 | 0.68 | 0.11 | 0.21 | 0.53 | 0.39 |
| 20:1(n-9) | 1.35 | 0.00 | 1.23 | 2.19 | 0.35 | 0.00 | 3.15 | 0.35 | 1.04 | 2.05 |
| 20:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:2(n-6) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:3(n-6) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-6) | 0.00 | 0.00 | 0.32 | 0.31 | 0.24 | 0.00 | 0.12 | 0.00 | 0.31 | 0.26 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.22 | 0.00 | 0.17 | 0.27 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-3) | 0.18 | 0.00 | 0.33 | 0.00 | 0.19 | 0.00 | 0.22 | 0.00 | 0.31 | 0.00 |
| 20:5(n-3) | 5.94 | 3.75 | 11.01 | 11.08 | 12.38 | 7.24 | 6.44 | 4.77 | 8.62 | 7.34 |
| 22:0 | 0.00 | 0.00 | 0.90 | 0.00 | 0.27 | 0.42 | 0.00 | 0.00 | 0.00 | 0.44 |
| 22:1(n-11) | 1.70 | 0.00 | 1.39 | 1.42 | 0.38 | 0.00 | 2.79 | 0.15 | 0.98 | 2.27 |
| 22:1(n-9) | 0.00 | 0.00 | 0.35 | 0.35 | 2.19 | 0.00 | 0.36 | 0.00 | 0.23 | 1.01 |
| 22:1(n-7) | 0.00 | 0.00 | 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 21:5(n-3) | 2.38 | 0.00 | 1.37 | 1.58 | 0.74 | 0.00 | 0.22 | 2.54 | 0.13 | 0.57 |
| 22:5(n-3) | 1.17 | 0.00 | 1.19 | 0.93 | 0.71 | 0.00 | 0.42 | 1 58 | 0.33 | 0.33 |
| 22:6(n-3) | 2.10 | 0.87 | 3.55 | 3.00 | 2.78 | 2.23 | 0.95 | 1.40 | 5.20 | 3.00 |
| 24:1 | 0.00 | 0.00 | 0.97 | 0.23 | 0.18 | 0.00 | 0.00 | 0.00 | 0.24 | 0.00 |
| FFA (ng $ml^{-1}$) | 1.1 | 3.1 | 5.1 | 5.2 | 3.7 | 13.1 | 10.9 | 5.1 | 4.6 | 3.2 |
| TL (ng $ml^{-1}$) | 7.2 | 5.0 | 19.4 | 15.3 | 8.5 | 22.8 | 14.1 | 5.0 | 7.7 | 6.0 |

Appendix 14. Continued.

| Fatty acid (%) | 54b | 58 | 66 | 76 |
|---|---|---|---|---|
| 14:0 | 7.04 | 7.53 | 10.98 | 21.71 |
| 14:1 | 0.24 | 0.00 | 0.65 | 1.01 |
| i-15:0 | 1.50 | 3.67 | 1.36 | 1.85 |
| ai-15:0 | 0.14 | 1.34 | 0.60 | 1.83 |
| 15:0 | 0.47 | 1.48 | 1.20 | 3.18 |
| 15:1 | 0.24 | 0.00 | 0.78 | 1.89 |
| i-16:0 | 0.32 | 0.54 | 1.48 | 0.47 |
| ai-16:0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:0 | 9.35 | 15.40 | 19.39 | 34.87 |
| 16:1(n-9) | 0.00 | 0.00 | 0.55 | 0.00 |
| 16:1(n-7) | 25.08 | 19.07 | 16.09 | 1.80 |
| 16:1(n-5) | 0.56 | 0.77 | 0.54 | 0.00 |
| i-17:0 | 0.36 | 1.19 | 0.78 | 2.68 |
| ai-17:0 | 0.15 | 0.00 | 0.42 | 0.62 |
| 16:2(n-4) | 0.95 | 0.57 | 2.02 | 2.47 |
| 16:2 | 0.33 | 0.71 | 0.00 | 0.00 |
| 17:0 | 0.70 | 0.00 | 0.75 | 0.97 |
| 16:3(n-4) | 1.17 | 1.53 | 0.92 | 2.62 |
| 17:1 | 0.00 | 0.00 | 0.74 | 0.00 |
| 16:3 | 0.14 | 0.00 | 0.44 | 1.59 |
| 16:4(n-3) | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:4(n-1) | 1.64 | 0.68 | 1.89 | 2.79 |
| 18:0 | 1.68 | 4.40 | 6.93 | 8.46 |
| 18:1(n-9) | 16.51 | 5.77 | 5.46 | 1.16 |
| 18:1(n-7) | 1.80 | 1.42 | 1.20 | 0.00 |
| 18:1(n-5) | 0.29 | 0.00 | 0.00 | 0.00 |
| 18:2(n-6) | 1.58 | 1.71 | 1.75 | 0.00 |
| 18:2(n-4) | 0.83 | 2.16 | 2.76 | 4.81 |
| 18:3(n-6) | 0.20 | 0.57 | 0.90 | 0.00 |
| 18:3(n-4) | 0.41 | 0.00 | 0.00 | 0.00 |
| 18:3(n-3) | 0.36 | 0.43 | 0.47 | 0.00 |
| 18:4(n-3) | 1.75 | 3.13 | 1.45 | 0.00 |
| 18:4(n-1) | 0.17 | 0.00 | 0.00 | 0.00 |
| 20:0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:1(n-11) | 0.22 | 1.96 | 1.05 | 0.00 |
| 20:1(n-9) | 3.37 | 1.62 | 0.95 | 0.00 |
| 20:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:2(n-6) | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:3(n-6) | 0.00 | 0.00 | 0.00 | 0.00 |
| 20:4(n-6) | 0.31 | 0.00 | 0.00 | 0.00 |
| 20:3 (n-3) | 0.00 | 0.00 | 0.45 | 0.00 |
| 20:4(n-3) | 0.16 | 0.94 | 0.00 | 0.00 |
| 20:5(n-3) | 11.54 | 10.51 | 7.23 | 1.89 |
| 22:0 | 0.00 | 0.00 | 1.48 | 0.00 |
| 22:1(n-11) | 2.50 | 0.00 | 0.00 | 0.00 |
| 22:1(n-9) | 0.16 | 2.30 | 0.00 | 0.00 |
| 22:1(n-7) | 0.00 | 0.00 | 0.00 | 0.00 |
| 21:5(n-3) | 0.82 | 1.34 | 1.66 | 0.00 |
| 22:5(n-3) | 0.61 | 0.00 | 1.76 | 0.00 |
| 22:6(n-3) | 4.18 | 7.27 | 2.92 | 1.33 |
| 24:1 | 0.14 | 0.00 | 0.00 | 0.00 |
| TFA (ng $ml^{-1}$) | 7.2 | 1.5 | 1.8 | 5.1 |
| TL (ng $ml^{-1}$) | 20.0 | 7.1 | 3.7 | 14.7 |

Appendix 15. All variables included in PCA analyses (Chapter 5): C.h.=*Calanus hyperboreus* CV; C.g.=*Calanus glacialis* CV; M.l.=*Metridia longa* females; Surf.=surface; other abbreviations on page x.

| Variable[a] | C.h. Surf. | C.h. Deep | C.g. Surf. | C.g. Deep | M.l. Deep |
|---|---|---|---|---|---|
| 14:0 | X | X | X | X | X |
| 14:1 | X | X | X | X | X |
| i-15:0 | X | X | X | X | X |
| ai-15:0 | X | | X | X | |
| 15:0 | X | X | X | X | X |
| 15:1 | X | X | X | X | X |
| i-16:0 | | | X | X | |
| ai-16:0 | | | | | |
| 16:0 | X | X | X | X | X |
| 16:1(n-9) | | | | | |
| 16:1(n-7) | X | X | X | X | X |
| 16:1(n-5) | X | X | X | X | X |
| i-17:0 | | | X | X | X |
| ai-17:0 | X | X | X | X | X |
| 16:2(n-4) | X | X | X | X | X |
| 16:2? | | | | | |
| 17:0 | | | | | |
| 16:3(n-4) | X | X | X | X | X |
| 17:1 | X | X | X | | |
| 16:3? | | | | | |
| 16:4(n-3) | X | X | X | X | |
| 16:4(n-1) | X | X | X | X | X |
| 18:0 | X | X | X | X | X |
| 18:1(n-9) | X | X | X | X | X |
| 18:1(n-7) | X | X | X | X | X |
| 18:1(n-5) | X | X | X | X | X |
| 18:2(n-6) | X | X | X | X | X |
| 18:2(n-4) | X | X | X | X | X |
| 18:3(n-6) | X | X | X | X | X |
| 18:3(n-4) | X | | | X | X |
| 18:3(n-3) | X | X | X | X | X |
| 18:4(n-3) | X | X | X | X | X |
| 18:4(n-1) | X | X | X | X | X |
| 20:0 | X | X | X | X | X |
| 20:1(n-11) | | | | X | |
| 20:1(n-9) | X | X | X | X | X |
| 20:1(n-7) | X | X | X | X | X |

| Variable[a] | C.h. Surf. | C.h. Deep | C.g. Surf. | C.g. Deep | M.l. Deep |
|---|---|---|---|---|---|
| 20:2(n-6) | X | X | | X | X |
| 20:3(n-6) | X | X | X | X | |
| 20:4(n-6) | X | X | X | X | X |
| 20:3(n-3) | | X | | | |
| 20:4(n-3) | X | X | X | X | X |
| 20:5(n-3) | X | X | X | X | X |
| 22:0 | | | | | |
| 22:1(n-11) | X | X | X | X | X |
| 22:1(n-9) | X | X | X | X | X |
| 22:1(n-7) | X | X | X | X | |
| 21:5(n-3) | X | X | X | X | X |
| 22:5(n-3) | X | X | X | X | X |
| 22:6(n-3) | X | X | X | X | X |
| 24:1 | X | X | X | X | X |
| ΣOBFA | X | X | X | X | X |
| ΣSFA | X | X | X | X | X |
| ΣMUFA | X | X | X | X | X |
| ΣPUFA | X | X | X | X | X |
| 16:1(n-7)/16:0* | X | X | X | X | X |
| DHA/EPA* | X | X | X | X | X |
| (n-3)/(n-6)* | X | X | X | X | X |
| UC* | X | X | X | X | X |
| HC | X | X | X | X | X |
| ME | | | | X | |
| KET | | | | | |
| WE | X | X | X | X | X |
| TG | X | X | X | X | X |
| FFA | | | | | |
| ST | | X | | | X |
| ALC | | | | | |
| DG | | | | | |
| AMPL | X | X | X | X | X |
| PL | X | X | X | X | X |
| %LIPID | X | X | X | X | X |
| WE/DM (μg/mg) | X | X | X | X | X |
| TFA (μg $cope^{-1}$) | X | X | X | X | X |
| TL (μg $cope^{-1}$) | X | X | X | X | X |
| DM (mg) | X | X | X | X | X |

[a]all variables expressed as percentages except otherwise noted: *unitless

# Memorial
University of Newfoundland

This is to authorize the Dean of Graduate Studies to deposit two copies of my thesis/report entitled

Lipid-Based Assessments of Omnivory in Arctic Copepods

in the University Library, on the following conditions. I understand that I may choose only ONE of the Options here listed, and may not afterwards apply for any additional restriction. I further understand that the University will not grant any restriction on the publication of thesis/report abstracts.

*(After reading the explanatory notes at the foot of this form, delete TWO of (a), (b) and (c), whichever are inapplicable.)*

The conditions of deposit are:

(a) that two copies are to be made available to users at the discretion of their custodians,

OR

(b) that access to, and quotation from, this thesis/report is to be granted only with my written permission for a period of one year from the date on which the thesis/report, after the approval of the award of a degree, is entrusted to the care of the University, namely, January 2004, after which time the two copies are to be made available to users at the discretion of their custodians,

OR

(c) that access to, and quotation from, this thesis/report is to be granted only with my written permission for a period of _____ years from the date on which the thesis/report, after approval for the award of a degree, is entrusted to the care of the University; namely, _____, 19___; after which time two copies are to be made available to users at the discretion of their custodians.

Date 18 August 2003

Signed Catherine Stern

Dean of Graduate Studies

Witnessed by _____

*NOTES*

1. *Restriction (b) will be granted on application, without reason given.*

   *However, applications for restriction (c) must be accompanied with a detailed explanation, indicating why the restriction is thought to be necessary, and justifying the length of time requested. Restrictions required on the grounds that the thesis is being prepared for publication, or that patents are awaited, will not be permitted to exceed three years.*

   *Restriction (c) can be permitted only by a Committee entrusted by the University with the task of examining such applications, and will be granted only in exceptional circumstances.*

2. *Thesis writers are reminded that, if they have been engaged in contractual research, they may have already agreed to restrict access to their thesis until the terms of the contract have been fulfilled.*